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STUDIES IN TREE PHYSIOLOGY

II. SEASONAL CHANGES IN THE FOOD RESERVES OF FIELD BIRCH (*BETULA POPULIFOLIA* MARSH.)[1]

BY R. DARNLEY GIBBS[2]

Abstract

During the period January 1936 to July 1938, the upper parts of *Betula populifolia* Marsh. were analysed for water, reducing sugars, sucrose, total soluble sugars, ether-soluble material, polysaccharide hydrolysable by taka-diastase, and residual polysaccharides extracted by dilute acid. From 38 to 63% of the taka-diastase hydrolysate from the wood is non-fermentable, presumably pentose. The residual acid extract is from 69 to 100% non-fermentable. Hexosan is low in winter, rises to a maximum just before leaf-opening, decreases sharply during spring growth and accumulates in summer. Ether-soluble material is not stored in winter. Sucrose and to a lesser extent reducing sugars are high in winter. There is a significant correlation between reducing sugars and water content of the twigs. Food reserves of the cortex behave much as do those of wood.

Introduction

In this paper, the second of a projected series on tree physiology, are recorded the results of analyses extending over two and a half years (from January 1936 to July 1938) of the upper parts of field birch. The trees used were young specimens, 15 to 20 ft. high and with a D.B.H. of 2 to 4 in., growing in a field (which they had invaded) at Ste. Anne de Bellevue, about 20 miles from Montreal. The water contents of these and other trees were reported in the first paper of this series (8).

The seasonal changes in deciduous trees in Eastern Canada are very marked. There is almost continuous frost from November until April and this is reflected in the "dormant" condition of the trees during this period. Leaves expand in early May and are dropped in October. Food manufactured in the leafy season is stored within the tree during the winter, to be used with the onset of growth in late April. This storage of food in winter is only one aspect of the physiology of the tree, for correlated with this is an increase in winter hardiness which is of great interest and practical importance. Stored foods, of course, are not alone responsible for this hardiness, but they are a part of the mechanism and must be included in any complete picture.

Schultz (17), in 1888, found that starch disappears from evergreen leaves in winter, to be replaced by tannin-like materials. Suroz (20), a little later, believed starch to be replaced by oil while Fischer (7), in the same year,

[1] *Manuscript received in original form May 10, and as revised October 31, 1939.*

Contribution from the Department of Botany, McGill University, Montreal, Que., with financial assistance from the National Research Council of Canada.

[2] *Assistant Professor of Botany, McGill University.*

published an extensive paper in which he classed trees as starch- and fat-trees, the former retaining much starch in winter, the latter replacing starch by fat. Fischer considered that most of the trees with hard wood belong to the first class, while those with softer wood, and the conifers, belong to the second. His conclusions have been criticized more recently by Preston and Phillips (16). Lidforss (12, 13) first began to correlate seasonal, food-reserve changes in evergreens with resistance to winter cold.

Most of the work that has been done in this field (and it is very extensive) has been only roughly quantitative, depending in great measure upon staining of microscopic sections for starch, hemicelluloses, and fats. This is true even of such recent work as that of Ishibe (10) who stained with Sudan III in a mixture of propyl alcohol and glycerine for "fats". It is very doubtful, as Doyle and Clinch pointed out some years ago (6), if the materials staining with osmic acid, Sudan III, and other so-called "fat" stains are really fats. Doyle has again drawn attention to this in a recent note (5).

There are several quantitative methods available for starch, though the accuracy of some of them may be questioned. It is not intended to deal with these in detail. The investigation of hemicelluloses is not an easy matter and it is doubtful if an exact characterization of hemicellulose fractions can yet be made.

Materials and Methods

In the present work the upper parts, consisting of one-, two-, and three-year-old twigs, of field birch were employed. Except in some of the later analyses the wood alone was used. In these last, however, the material was separated into wood and "cortex" (all outside the wood) and both were analysed. Material was collected in each case from three trees, samples being taken for determination of water at the same time (8). These were dried at 105° C. in an electric oven, which has been shown to give accurate results with wood of birch (9). The twigs were brought quickly into the laboratory, wood and cortex separated as rapidly as possible, and the wood roughly ground in a pencil sharpener. The ground material was then sampled into four lots with a Jones sampler; the lots, each of about 10 gm., were weighed, and two were dried at 105° C. in an electric oven, while the others were used at once for analysis. This second water determination permits of the reference of all figures to a dry weight basis.

The fresh samples were thrown into boiling alcohol to which a pinch of precipitated calcium carbonate had been added. The calcium carbonate was supposed to neutralize any acids present and so to prevent hydrolysis of any sugars, but in a paper which was brought to our attention after our analyses were started Denny (2) questioned the use of carbonate in this connection. However, since carbonate was added in the early analyses its use was continued throughout the series. The alcohol employed was of sufficient strength to give, with the water in the tissue, a final strength of about 80%. It was poured off and the extraction was repeated several times.

The samples were then transferred to a small Pyrex glass ball-mill and ground in 95% alcohol for 6 to 7 hr. The alcohol was filtered off under suction, the cake broken up and extracted exhaustively with ether in an all-glass Soxhlet apparatus having "standard-taper" joints.

The alcoholic extracts were combined and the alcohol evaporated off under reduced pressure. The residual liquid (mostly water) contained the soluble sugars and a scum of fat, pigments, etc., which had been extracted by the alcohol. The mixture was therefore thoroughly ether-extracted by repeated shaking in a separatory funnel.[1] The ether extracts so obtained were added to the Soxhlet extract, and the whole evaporated down and dried at 100° C. as *ether soluble material*. It consisted, of course, of much more than true fat, but was not further differentiated.

The aqueous residue from the separatory-funnel extractions contained the soluble sugars. It was cleared by neutral lead acetate followed by potassium oxalate[2] and aliquots were analysed for *reducing sugars* by the method of Shaffer and Somogyi (18).

Further aliquots were quantitatively inverted by "convertit", a commercial invertase preparation[3], and analysed for *total soluble sugars*. By difference, values for *sucrose* were obtained. As will be seen later, field birch contains considerable quantities of pentosans, and it was thought that some pentose might be contained in these soluble sugar fractions. The amount, however, proved to be quite low, for the solutions gave only weak reactions for pentoses and were almost quantitatively fermentable by yeast.

The cake of ground material left after the Soxhlet extraction was dispersed in water and heated to drive off the ether and to swell and disintegrate the starch grains in the tissue fragments (3). The mixture was then buffered to approximately pH 4.5 with acetic acid plus sodium acetate and treated with special undiluted taka-diastase[4] in an incubator at 37°C. for two days, a little toluol being added as an antiseptic. A second taka-diastase treatment for two days followed and the extracts were combined, boiled, and filtered. The filtrate was almost colourless but somewhat cloudy. The sugar in this solution was estimated as above, and at first assumed to be derived entirely from *"starch"*; but it was found that strong pentose reactions were given, and careful fermentations showed that a considerable quantity—up to 65%—of this material was not fermentable (see also (3, 4) in this connection). Fermentation was therefore adopted as a standard procedure, and the reducing substances were differentiated into "hexose" and "pentose". Pentoses have not the same reducing power as hexoses with the carbonate-citrate reagent employed, but a few analyses of known pentose solutions show that the difference is comparatively small. The ball-mill grinding mentioned above is necessary only for the taka-diastase digestions. We therefore attempted

[1] *A continuous extraction apparatus was used for a while but found unsatisfactory.*

[2] *During the period October 1936 to March 1937, duplicate samples were cleared with "Norit" charcoal and analysed for sugars. The values obtained were sometimes considerably lower than those from lead-cleared extracts.*

[3] *Eimer and Amend, N.Y.*

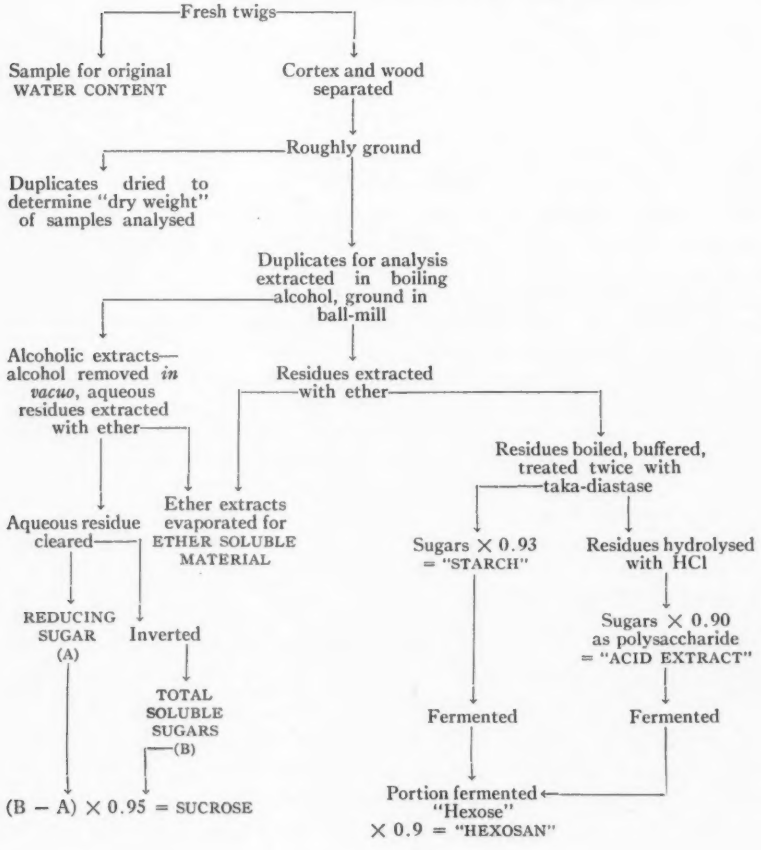
[4] *Takamine Laboratory, Inc., Clifton, N.J.*

to combine the grinding and digestion by delaying the former until after ether extraction, and then incubated with taka-diastase while grinding. This, however, was found to give very low results and was abandoned in favour of the procedure outlined above.

The residue from taka-diastase digestion was now treated at 100° C. for one hour with 2% hydrochloric acid. This was designed to check the completeness of extraction with taka-diastase and was found by fermentation to yield a considerable amount of pentose but very little (or sometimes no) hexose. We have, therefore, combined the values for "hexose" obtained by taka-diastase and acid extraction as "*Hexosan*" (chiefly starch?). The whole scheme is summarized in the flowsheet that follows.

FLOWSHEET

SCHEME OF ANALYSIS FOR TWIGS OF FIELD BIRCH


Fresh twigs
Sample for original WATER CONTENT
Cortex and wood separated
Roughly ground
Duplicates dried to determine "dry weight" of samples analysed
Duplicates for analysis extracted in boiling alcohol, ground in ball-mill
Alcoholic extracts—alcohol removed *in vacuo*, aqueous residues extracted with ether
Residues extracted with ether
Residues boiled, buffered, treated twice with taka-diastase
Aqueous residue cleared
Ether extracts evaporated for ETHER SOLUBLE MATERIAL
Sugars × 0.93 = "STARCH"
Residues hydrolysed with HCl
REDUCING SUGAR (A)
Inverted
Sugars × 0.90 as polysaccharide = "ACID EXTRACT"
TOTAL SOLUBLE SUGARS (B)
Fermented
Fermented
(B − A) × 0.95 = SUCROSE
Portion fermented "Hexose" × 0.9 = "HEXOSAN"

Results

Sucrose shows a regular variation during the year, rising to a maximum in December and remaining constantly high until March (Fig. 1). The maximum in the winter of 1936–7 was 2.9%, in 1935–6 it was probably about the same (2.9% in February 1936), while in the corresponding period for 1937–8 it was distinctly higher at 3.8%. A rapid decrease occurs in March-April and this decrease precedes the uptake of water, a quite interesting point. The lowest concentrations are reached in May, when leaves are expanding and growth is very rapid. The minima for 1936, 1937, and 1938 respectively were 0.5%, 0.7%, and 1.0%. The sucrose content increases slowly during the summer, until in late October it may be from 0.8% (1936) to 1.2% (1937). There is then the rapid increase to the high values noted for the winter.

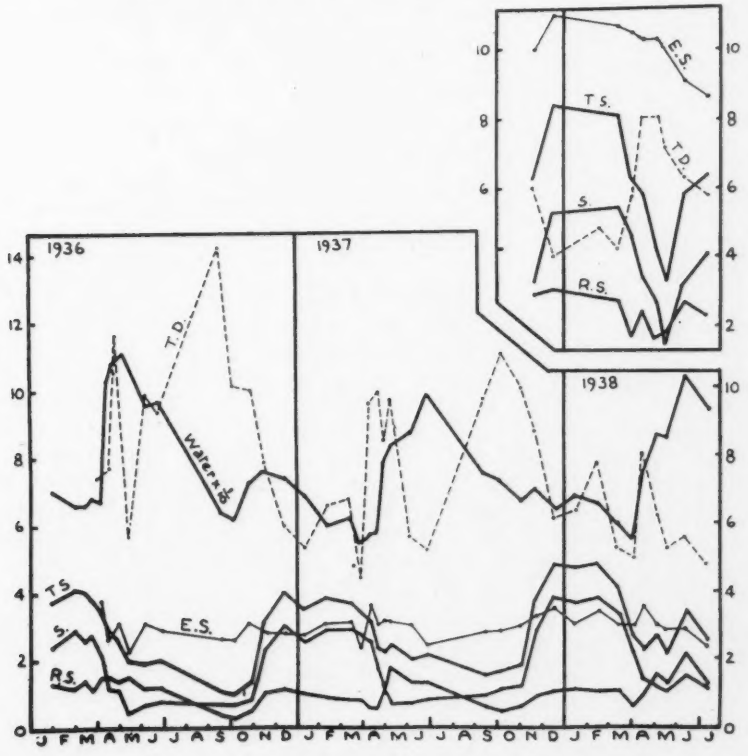


FIG. 1. *Seasonal changes in food reserves of field birch; wood below, cortex above.* R.S.= *reducing sugars;* S=*sucrose;* T.S.= *total soluble sugars;* E.S.= *ether-soluble material;* T.D.=*sugar obtained by taka-diastase hydrolysis calculated as "starch". The fluctuations in water content of wood are also included.*

Reducing sugars are never present in such large amounts as is sucrose nor are their fluctuations quite as regular. Values are relatively high in the winter, with minima in October and in late March or early April. The behaviour in spring seems to vary greatly from year to year. In April and May of 1936, concentrations were not markedly higher than during the winter, but in May, 1937 and in May and June of 1938 sharp increases occurred. These started from lower levels than in 1936 and reached higher maxima (1.1 to 1.5, 0.6 to 1.7, 0.6 to 2.1 in the respective years). These high spring concentrations coincided with low values for sucrose and might be due to inversion of the latter. Unlike sucrose, which increased slowly during the two summers studied, the reducing sugars decreased to very low levels in October (0.3% in 1936, 0.5% in 1937). An interesting point is the statistically significant correlation between water content and amount of reducing sugars based on dry weight (correlation coefficient = 0.66 ± 0.091). This does not mean a constant concentration of sugar in the water present, however, for actually there is a variation on this basis of from 0.5 to 2.2% in the period studied and the highest concentrations occur in winter (when water is low) and at leaf-opening (when water is high).

The curve for total soluble sugars shows a high value in winter and a low one in summer, with a minimum in September or October.

The behaviour of soluble sugars in the cortex is very similar to that in the wood (Fig. 1). During the winter sucrose is present in amounts much above that of the reducing sugars, but decreases sharply in spring to a value below them.

The present investigation tells little about the distribution of the sugars in the tissues studied, but we may hazard a guess that in the cortex most of the sugar is in the living cells while in the wood much of it is in the extra-cellular water. This is based upon a comparison of our figures with those for cortical cells of certain species given by Levitt and Scarth (11). These authors found, in their studies on frost-hardening, that the osmotic pressures of living cortical cells of *Caragana arborescens*, *Liriodendron tulipifera*, *Catalpa hybrida*, *Hydrangea paniculata*, and *Picea pungens* follow the curve given in Fig. 2 (we have averaged their results for these five plants). If we assume that living cells of the wood of field birch behave similarly this would account for only a very small amount of sugar (since the wood contains relatively few living cells). Actually we find that there is enough sugar in birch wood to give osmotic pressures, with all the water present, of as much as one-fifth of those found by Levitt and Scarth. Much of this sugar, then, is probably in the extra-cellular water. This is, perhaps, supported by the observation of the correlation between water content and reducing sugars already noted.

In cortex, on the other hand, living cells occupy a much higher proportion of the tissues. Unfortunately we have no accurate figures for water content of cortex so no direct comparisons of concentration can be made, but water contents of the samples as prepared for analysis suggest that changes in water content of fresh material were such that we could construct a curve for osmotic

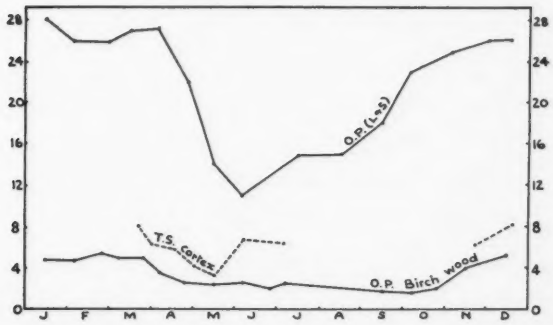


FIG. 2. *Upper curve—osmotic pressures in atmospheres of the cortical cells of hardy woody plants (Levitt and Scarth). Lower curve—osmotic pressures of soluble sugars in wood of field birch (on the assumption that the sugars are evenly distributed in the water present). Middle curve—total soluble sugars in cortex of field birch (percentage based on dry weight).*

pressure in this case which would lie slightly below that given by Levitt and Scarth but would follow essentially the same course. This would mean that much of the sugar present is in the living cells.

The ether-soluble material varies but little in amount throughout the year in the wood of field birch and is not significantly higher during the winter (Fig. 1). There is, then, no evidence here of fat storage in winter. This is not in agreement with the results of Niklewski (14) for wood of *Betula alba*, since he found decidedly more "fat" (ether extract) in late October and January than in August and February. There is some evidence of a high ether-extract from cortex of field birch in winter (Fig. 1) but our analyses for this part of the tree do not cover a sufficiently long period for any generalizations to be made. It is hoped, in later work, further to characterize the ether-extract.

Turning now to the material hydrolysed by taka-diastase—which might be listed as starch, but that it is only partially fermentable—we find in the wood great variations from year to year, but a general picture recalling that of Fischer (*loc. cit.*) for starch. Comparatively low values are found in winter. There is then a sharp increase just before leaf opening (not well marked in 1938) and an equally sharp decrease during the early stages of growth. Sugar changes seem not to be sufficient to account for the spring increase, but it must be remembered that we have taken no account of possible translocation in the present investigation. Storage of material hydrolysable by taka-diastase occurs during summer and the highest recorded values are reached in September or early October. There is then a rapid decrease which coincides with the increase in soluble sugars.

When the taka-diastase hydrolysate is fermented there is a fairly close correlation between the amount of hydrolysate and the proportion fermented (correlation coefficient = 0.54 ± 0.176)—*i.e.*, the non-fermentable ("pentose") proportion is not varying as much as the fermentable ("hexose") fraction.

An essentially similar relation is seen, as far as our figures go, in the cortex, with a low "starch" value in winter, a rapid increase in spring and a sharp decline following leaf-opening. The correlation between taka-diastase hydrolysate and proportion of hydrolysate fermentable is even closer in the cortex than it is in the wood.

The acid extract was used, as noted, to test the completeness of extraction of hexosan by taka-diastase, and it is probable that no very definite fraction is extracted here, at least under the condition of the experiments. Usually it is almost all pentose.

When the "hexose" fractions of the taka-diastase and acid hydrolysates are combined we get figures for easily-extracted "*hexosan*" (probably mostly starch), which show the type of seasonal variation which Fischer believed to be characteristic of starch in many trees (Fig. 3). The close parallel between the hexosan and taka-diastase hydrolysate curves make it possible to use the latter as a measure of "starch" content.

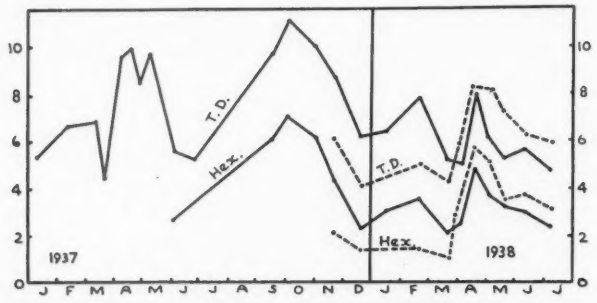


FIG. 3. *Seasonal changes in "polysaccharides" of field birch.* Hex. = *"hexosan"*; T.D. = *sugar obtained by taka-diastase hydrolysis, calculated as "starch". Solid lines for wood, dotted for cortex.*

One last point which is of considerable interest may be noted here. In trying to explain the seasonal changes in food reserves of trees, Sinnott (19) suggested that ease of access of water to storage cells might be a great factor, possibly through carriage of enzymes. A consideration of our curves for water content and starch, however, shows that the formation of starch in spring is quite independent of changes in water content. Thus, in 1936 a marked increase in starch occurred some time after the spring increase in water content, in 1937 it occurred well before any seasonal change, and in 1938 practically coincided with it.

Acknowledgments

The author wishes to acknowledge the kindness of Mr. Cleveland Morgan in permitting the use of trees from his land at Ste. Anne de Bellevue. Dr. F. E. Denny, of the Boyce Thompson Institute, provided valuable information on methods for the estimation of starch, while Dr. S. C. Reed assisted with calculations of significance and correlation. Our thanks are tendered to them all.

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NEW SPECIES OF GYMNOSPORANGIUM[1]

By Ivan H. Crowell[2]

Abstract

Gymnosporangium minus n. sp. occurs on *Cupressus sempervirens* L. near Athens, Greece, and is the only species on a non-juniperus telial host in Europe. *G. guatemalianum* n. sp. was found on *Amelanchier nervosa* (Dcne.) Standl. and *G. meridissimum* n. sp. on *Cupressus benthami* Endl. in Guatemala; they are the most southerly species of the genus and may be genetically related.

1. *Gymnosporangium minus* Crowell, sp. nov., III (Fig. I).

Pycnidia et aecidia ignota. Uredinia desunt. Soris teleutosporiferis caulicolis, pulvinatis, flavobrunneis vel castaneobrunneis; teleutosporis ellipsoideis, medio 1-septatis, rarius non-septatis, leviter constrictis mediis, 17.5 – 24.5μ × 45.5 – 59.5μ plus minusve 22.3μ × 50.4μ, quaque cellula poris germinationis 2 prope septum, pedicello brevi, hyalino.

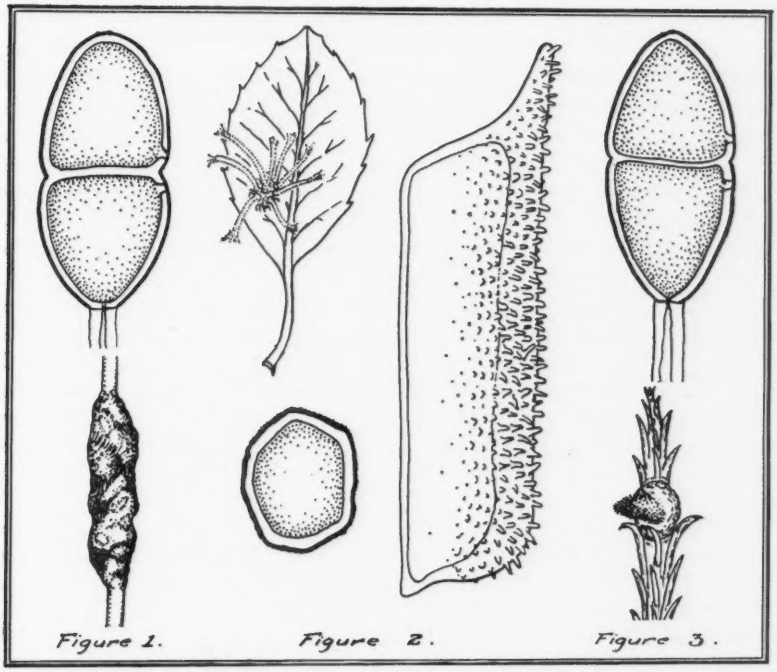
Habitat: soris teleutosporiferis in *Cupressus sempervirens L.* "Collected near Athens, on the road to Aleusis, Greece," April 3, 1906. A. S. Pease No. 8924½. Type specimen deposited in the Farlow Herbarium of Harvard University.

Although several species of *Gymnosporangium* attack a group of closely related telial hosts, many are confined to a single species; one only, namely, *G. clavipes*, parasitizes hosts in two sections of the genus *Juniperus*, but no species is known to attack hosts in more than a single genus of telial hosts. Furthermore, all these rusts on non-juniperus telial hosts, as *Cupressus*, are of local distribution. *G. minus* is the only species of *Gymnosporangium* on a non-juniperus telial host recorded from Europe.

In Oudeman's Enumeratio Systematica Fungorum 1: 663, 1919, *G. sabinae* (Dicks.) Wint. is reported on *Cupressus sempervirens*, but additional data are not given. In view of the host specificity in the genus *Gymnosporangium* and the lack of cultural data to prove otherwise, it appears that *G. minus* is a valid species. It is assumed that an error was made in the determination of *G. sabinae* as occurring on *Cupressus sempervirens*. Kern (1911) in his "A Biologic and Taxonomic Study of the Genus Gymnosporangium" states that teliospores of *G. sabinae* measure 19–30μ × 37–48μ, and are usually narrowed at both ends. Teliospores of *G. minus* are by my own comparisons narrower, considerably longer and more rotund terminally than those of *G. sabinae*.

[1] *Manuscript received August 29, 1939.*
Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que. Macdonald College Journal Series No. 132.

[2] *Lecturer in the Department of Plant Pathology, Macdonald College, Que.*



FIGS. 1–3. FIG. 1. *Gymnosporangium minus Crowell, n. sp.* FIG. 2. *Gymnosporangium guatemalianum Crowell, n. sp.* FIG. 3. *Gymnosporangium meridissimum Crowell, n. sp.*

2. *Gymnosporangium guatemalianum* Crowell sp. nov., O-I (Fig. 2).

Spermogonia foliicolae, subepidermalia. Aecidia hypophylla, roestelioidea, tumoribus tumidis insidentia 5–8 mm. alta, 0.25–0.5 mm. lata, cellulae peridii elongatae, hyalinae, pariete interiore spinulosae, marginibus gradatim plus breviter spinulosae, 24–32μ × 80–120μ plus minusve 27.6μ × 106.8μ; aecidiosporae globosae, pallide brunneae, verruculosae, 20–24μ × 24–30μ, plus minusve 21.4μ × 26.4μ.

Habitat: in foliis *Amelanchier nervosa* (Dcne.) Standl. in Parramos, Guatemala, December 14, 1938, J. R. Johnston No. 1444. Uredinia desunt. Soris teleutosporiferis ignota.

Type specimen deposited in Arthur Herbarium, Purdue University, Lafayette, Indiana. Co-type in Herbaria de la Escuela Nacional Central de Agricultura, Chimaltenango, Guatemala, and Macdonald College Mycological Herbarium, Macdonald College, Que.

3. *Gymnosporangium meridissimum* Crowell, sp. nov., III (Fig. 3).

Pycnidia et aecidia ignota. Uredinia desunt. Soris teleutosporiferis caulicolis, pulvinatis, flavobrunneis vel castaneobrunneis; teleutosporis ellip-

soideis, medio 1-septatis, leviter constrictis mediis, 24–28μ × 48–60μ plus minusve 25.6μ × 51.8μ, quaque cellula poris germinationis 2 prope septum, aliquando 1 prope septum et 1 prope apex, pedicello longi, hyalino.

Habitat: soris teleutosporiferis in *Cupressus benthami* Endl. = *C. lusitanica* Mill. in Alameda, Guatemala, April 1937. J. R. Johnston. Also found in Antiqua, Guatemala.

Type specimen deposited in Arthur Herbarium, Purdue University, Lafayette, Indiana. Co-types in Herbario de la Escuela Nacional Central de Agricultura, Chimaltenango, Guatemala, and Macdonald College Mycological Herbarium, Macdonald College, Que.

Small, rotund galls with one or two telia are produced on the young twigs and bear the first crop of spores. Most of these galls die shortly afterwards, but infections occasionally persist and form characteristic, deeply-fissured, fusiform swellings on lateral branches and the main trunk. These swellings vary in size up to approximately a foot in length. This is the only species of *Gymnosporangium* on a non-juniperus telial host known to form rotund galls. *G. guatemalianum* and *G. meridissimum* are possibly different stages of the same species.

The Guatemalian stations for these species of *Gymnosporangium* are the most southerly on record for the genus. *G. clavipes* found near Mexico City, Mexico, is the only other species reported south of the United States in continental North America. The hosts for these species, as well as for the one from Europe, are new for the genus *Gymnosporangium*.

VEGETATIVE PROPAGATION OF CONIFERS

IV. EFFECTS OF CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID IN TALC DUST ON THE ROOTING OF NORWAY SPRUCE[1]

By N. H. Grace[2]

Abstract

Norway spruce cuttings collected in November from the upper part of the tree were treated with a series of talc dusts containing indolylacetic and naphthylacetic acids, each at 0, 1000, and 5000 p.p.m., combined with cane sugar at 0 and 10%, and ethyl mercuric phosphate at 0 and 50 p.p.m., and propagated in a greenhouse. Indolylacetic acid at 1000 p.p.m. increased rooting by about 10% to 42.5%, three months after planting, but at 5000 p.p.m. reduced it significantly. Naphthylacetic acid reduced rooting at both concentrations. Indolylacetic acid increased the length of root per rooted cutting. Sugar in combination with indolylacetic acid inhibited the increase in root length caused by the hormone alone; organic mercury alone or in combination with indolylacetic acid reduced root length, but sugar and mercury in combination exerted no greater inhibition than either alone.

Mean root length was affected only by indolylacetic acid, which increased it.

Previous studies have shown that treatment of plant cuttings with a series of talc dusts containing indolylacetic acid, cane sugar, and ethyl mercuric phosphate may be advantageous (4). The present communication describes an experiment in which dormant Norway spruce cuttings, collected in November, 1938, were treated with a similar series of dust preparations.

Experimental

The experiment was of factorial design, comprising treatments with indolylacetic and naphthylacetic acids each at three levels, namely, 0, 1000, and 5000 p.p.m., in combination with cane sugar at 0 and 10%, and ethyl mercuric phosphate at 0 and 50 p.p.m.; the carrier dust was talc. The series of 20 treatment combinations of indolylacetic and naphthylacetic acids (separately) with cane sugar and organic mercury was included in each of six randomized blocks, the experimental unit being a group of 10 cuttings. There were thus six replicates, of which three were planted in sand and three in a mixture of sand and peat in equal proportions.

Branches were collected in November from the upper region of trees in a plantation of Norway spruce approximately 18 years of age and situated at the Petawawa Forest Experiment Station, Chalk River, Ontario. The bases of the branches were placed in moist peat, and they were stored outside until 21 December, 1938, when cuttings were prepared and treated.

[1] *Manuscript received November 21, 1939.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 872.

[2] *Biochemist.*

Cuttings ranged in length from 2 to 3½ in. and did not have a heel. Each group of 10 contained four short and three of each of two other length classes. There were approximately four laterals to one branch terminal cutting. The experiment required a total of 1200 cuttings. Groups of 10 cuttings were treated by dipping the basal ends in the various dust preparations to a depth of about one-half inch, and excess dust was shaken off. The cuttings were planted immediately in propagation frames equipped with bottom heat cables. Both media were maintained at about 72° F., while the greenhouse temperature was around 65° F. The frames were provided with factory cotton screens, which reduced light and maintained humidity. The cuttings were removed three months after planting.

Results

Records were made of the number of cuttings rooted, the number of roots and their length were determined, and the mean root length was calculated. The analysis of variance of number and lengths of root per rooted cutting, and mean root length, refer to treatments with 0 and 1000 p.p.m. indolylacetic

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS TO DUST TREATMENTS WITH 1000 P.P.M. INDOLYLACETIC ACID, 10% CANE SUGAR, AND 50 P.P.M. ETHYL MERCURIC PHOSPHATE

Treatments with (A) indolylacetic and naphthylacetic acids at 0, 1000, and 5000 p.p.m.; (B) indolylacetic acid at 0 and 1000 p.p.m.; cane sugar (0 and 10%) and organic mercury (0 and 50 p.p.m.) common to both A and B.

Source of variance	Degrees of freedom (A)	Mean square	Degrees of freedom (B)	Mean square		
	Number of cuttings rooted	Number of cuttings rooted†	Measurements on rooted cuttings	Number of roots per rooted cutting	Length of roots per rooted cutting	Mean root length
Between replicates						
Sand *vs.* peat-sand	1	3.9205	1	0.1875	0.75	17.16
Within media	4	1.0584	4	0.6238	607.57	137.01
Phytohormone treatment	4	5.4302***	1	0.0008	972.00**	239.86**
Sugar treatment	1	0.3381	1	0.1408	330.75*	49.82
Organic mercury treatment	1	0.0092	1	0.3008	374.08*	85.60
Interaction						
Phytohormone × sugar	4	0.1932	1	0.7008	850.08**	69.36
Phytohormone × organic mercury	4	0.1970	1	1.4008	270.75	5.14
Interaction						
Sugar × organic mercury	1	0.0126	1	0.2408	432.00*	53.13
Phytohormone × sugar × organic mercury	4	0.2044	1	0.3008	40.33	0.32
Error	95	0.1676	35	0.3658	79.84	24.08

† *Data transferred* $\sqrt{x + \frac{1}{2}}$ *basis for analysis of variance (1).*

* *Exceeds mean square error, 5% level of significance.*

** *Exceeds mean square error, 1% level of significance.*

*** *Exceeds mean square error, 0.1% level of significance.*

acid only. The poor rooting and numerous complete failures following treatments with 5000 p.p.m. indolylacetic acid and 1000 and 5000 p.p.m. naphthylacetic acid resulted in data unsuited to treatment by the analysis of variance procedure. The remaining data were subjected to analyses of variance with the results indicated in Table I. In Part A we see that hormone treatment had a highly significant effect on the number of cuttings rooted. None of the other treatments, nor the various interactions, attained significance. In Part B of the table we see there were no significant treatment effects on the number of roots per rooted cutting. However, length of root per rooted cutting was affected significantly by indolylacetic acid, cane sugar, and organic mercury, and there were two significant interactions, between indolylacetic acid and sugar, and between sugar and organic mercury. Mean root length was affected by indolylacetic acid—the only significant treatment effect on this measurement. In no instance did the difference between sand and peat-sand attain significance.

Table II shows the average effects, both with and without sugar and organic mercury, of indolylacetic and naphthylacetic acids, on the number of cuttings rooted. Treatment with 1000 p.p.m. of indolylacetic acid increased rooting significantly, while the 5000 p.p.m. level and both dosages of naphthylacetic acid significantly reduced rooting. Rooting at 5000 p.p.m. naphthylacetic acid was significantly below that following all other treatments.

TABLE II

ROOTING OF NORWAY SPRUCE CUTTINGS TREATED WITH INDOLYLACETIC AND NAPHTHYLACETIC ACIDS

Transformed data are means of 24 groups of 10 cuttings

	Concentration of chemical in talc, p.p.m.					Necessary difference, 5% level
	0	Indolylacetic acid		Naphthylacetic acid		
		1000	5000	1000	5000	
Cuttings rooted of 10 planted, transformed data†	1.835	2.140*	1.459*	1.450*	0.874*	0.235
Per cent of cuttings rooted	32.9	42.5	18.8	18.3	3.3	

* *Significantly different from control, 5% level.*

† *Data transformed to* $\sqrt{x + \frac{1}{2}}$ *basis for analysis of variance (1).*

In Table III are shown the average effects, with and without organic mercury, of indolylacetic acid and cane sugar on the length of root per rooted cutting. In the absence of sugar, indolylacetic acid increased the length of root, but the interaction between indolylacetic acid and sugar was such that the use of sugar effectively inhibited this stimulating effect of the hormone chemical.

TABLE III

ROOT LENGTHS PER ROOTED CUTTING (MM.) OF NORWAY SPRUCE CUTTINGS TREATED WITH DUSTS CONTAINING INDOLYLACETIC ACID AND CANE SUGAR

Cane sugar, %	Indolylacetic acid, p.p.m.		Means
	0	1000	
0	21	38	30
10	24	25	24
Means	23	32	

Necessary difference, 5% level, treatment means 5.2, interaction 7.4.

In Table IV are given the average effects, with and without indolylacetic acid, of organic mercury and cane sugar. In the absence of sugar, organic mercury at 50 p.p.m. reduced the length of root per rooted cutting. The addition of mercury in the presence of sugar made no difference to the extent to which root lengths were depressed. Thus, the two depressing influences together were no more effective than either alone.

TABLE IV

ROOT LENGTHS PER ROOTED CUTTING (MM.) OF NORWAY SPRUCE CUTTINGS TREATED WITH ORGANIC MERCURY AND CANE SUGAR

Organic mercury, p.p.m.	Cane sugar, %		Means
	0	10	
0	36	24	30
50	24	24	24

Necessary difference, 5% level, treatment means 5.2, interaction 7.4.

The mean length of individual roots after treatment with indolylacetic acid was 14.3 mm. as compared with 9.9 mm. without, 2.9 mm. being the necessary difference for the 5% level of significance. Treatment with 1000 p.p.m. indolylacetic acid at both dosages of cane sugar and organic mercury significantly increased the mean root length.

Discussion

Indolylacetic acid in talc dust at 1000 p.p.m. has increased the rooting of Norway spruce cuttings by about 10%. When 5000 p.p.m. of this chemical or 1000 and 5000 p.p.m. of naphthylacetic acid were used, rooting was reduced. Numerous experiments with spruces have demonstrated that it is difficult

to show improved rooting from the use of root-growth-stimulating chemicals, though injurious effects have been shown with comparative ease. A later paper will deal with the injurious effects of indolylacetic acid *solutions* on the rooting of Norway spruce cuttings.

In earlier experiments by the author (2, 3) treatment of Norway spruce cuttings with 1000 p.p.m. of indolylacetic acid in talc dust did not increase rooting significantly, though it did increase the number of roots per rooted cutting in one experiment and reduced the mean root length in two. Since the present experiment made use of material collected at the same time and propagated under closely similar conditions, a comparison of the results is justified. The sole apparent difference in procedure in the experiments was the use of cuttings with a heel in the former and plain cuttings in the present one. It is possible that differences in response to 1000 p.p.m. indolylacetic in talc may be attributed to the adhesion of a greater amount of dust to the cuttings with a heel, resulting in reduced mean root length and increased number of roots per rooted cutting. However, further experiments will be required to demonstrate whether this is so, or whether the differences in response of cuttings with and without a heel are physiological in nature.

The addition of sugar or organic mercury failed to affect the number of cuttings rooted. Some reduction in length of root per rooted cutting was noted when sugar was applied in addition to indolylacetic acid. Organic mercury also reduced root length with and without the presence of indolylacetic acid.

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THE SEXUAL BEHAVIOUR OF SEVERAL PLANT RUSTS[1]

By A. M. Brown[2]

Abstract

Experiments conducted under greenhouse conditions have shown that *Uromyces Trifolii hybridi*, *U. Fabae*, and *Phragmidium speciosum* are heterothallic. All of these rusts produce well developed pycnia. One of them, *Uromyces Fabae*, may shorten its life-cycle by omitting aecia or may produce uredia and aecia in association.

By similar experiments it has been found that *Puccinia coronata Elaeagni*, *P. Grindeliae* and *P. Xanthii* are homothallic. Ashworth's conclusion that *P. Malvacearum* is homothallic has been confirmed. In all of these rusts, single sporidia give rise spontaneously to binucleate infections. *P. Malvacearum*, *P. Xanthii*, and usually *P. Grindeliae* lack pycnia, while *P. coronata Elaeagni*, a rust described as being without pycnia, is shown to produce occasional ones.

Introduction

Since 1927, the year in which it was discovered that *Puccinia Helianthi* Schw. (11) and *P. graminis* Pers. (12) are heterothallic, the following species have been shown to possess a similar sexual behaviour: *P. triticina* Erikss. (1), *P. coronata* Corda. (2), *P. Sorghi* Schw. (4), *Melampsora Lini* (Pers.) Lev. (3), *Uromyces appendiculatus* (Pers.) Fries, *U. Vignae* Barclay (6), *Gymnosporangium Haraeanum* Syd. (17), *G. juniperi-virginianae* Schw. and *G. globosum* Farl. (19), *Puccinia Phragmitis* (Schum.) Körn. (18), *Cronartium ribicola* J. C. Fischer (23), *Uromyces graminis* (Niessl) Diet. (21), and *Puccinia anomala* Rostr. (22). Heterothallism is therefore a well known phenomenon in the rust fungi. Homothallism, however, up to the present seems to have been proved experimentally only in *P. Malvacearum* Bert. (7), and, in this case, doubt has been thrown upon the evidence (5). Jackson's (16) conclusion that, in this rust, "every infection, monosporidial or otherwise, develops one or more sori of teliospores" indicates very clearly that he found it to behave as a homothallic species.

As a result of the experiments now to be presented, it is possible to add to the above list of heterothallic species the following three rusts; *Uromyces Trifolii hybridi* (W. H. Davis) Arth., *U. Fabae* (Pers.) de Bary, and *Phragmidium speciosum* (Fries) Cooke. In addition to the heterothallic species just enumerated, three other species, *Puccinia coronata Elaeagni* F. and L., *P. Grindeliae* Peck,[3] and *P. Xanthii* Schw. are shown to be homothallic; and the finding of Ashworth (7) that *P. Malvacearum* is homothallic is confirmed.

The rust material made use of in this investigation was, with one exception, collected in the neighbourhood of Winnipeg, Manitoba. The exception was

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[3] *Arthur in his "Manual of the Rusts in the United States and Canada" indicates that on certain hosts, particularly Xylorrhiza, P. Grindeliae occasionally produces pycnia.*

P. coronata Elaeagni. Telia of this rust and roots of its aecial host, *Elaeagnus commutata* Bernh., for propagation purposes, were kindly provided by Dr. R. C. Russell, of the Dominion Laboratory of Plant Pathology, Saskatoon, Saskatchewan. An investigation of the sexual behaviour of *Puccinia coronata Elaeagni* was suggested by Dr. G. A. Ledingham, who pointed out that the absence of normal pycnial development in this variety might indicate a sexual behaviour different from that of *P. coronata Avenae* F. and L. which Allen (2) showed to be heterothallic.

Sporidial inoculation of these rusts was secured on their respective hosts by suspending germinating teliospores over seedling host-plants. This procedure, which usually provides a goodly number of well isolated monosporidial infections, is more fully outlined by Craigie (13) and Newton and Johnson (20). The procedure followed in diploidizing haploid infections of the pycnium-producing rusts by means of pycnial-nectar transfers, and by permitting haploid and diploid (uredial) infections to unite, has previously been described by Craigie (12) and Brown (9) respectively.

The experiments were, for the most part, conducted during the winter months, and during that time insects that might transfer nectar from one infection to another were practically absent from the greenhouses. However, in order to prevent any possibility of such a transfer, each plant was enclosed in a screen-wire cage.

Heterothallic Species

Uromyces Trifolii hybridi

As a result of suspending germinating teliospores of *U. Trifolii hybridi* over seedling plants of *Trifolium hybridum* L., 60 well-isolated haploid infections were secured. Of this number, 20 infections were allotted as controls; to 20 others, composite pycnial nectar collected from infections not used in the experiments was applied; and, near the periphery of 15 others, urediospores of this rust were sown. The plants bearing these 15 infections together with the remaining five haploid infections were enclosed in a moist chamber for 48 hr., a procedure usually followed in uredial inoculation practice. The five haploid infections served as a control for the other 15.

Of the 20 infections to which pycnial nectar was applied, 19 produced aecia within seven days following the application of nectar, but the 20 control infections, *i.e.*, those to which no nectar was applied, were still haploid at the termination of the experiment two weeks later. All of the 15 infections, around the periphery of which urediospores were sown, produced aecia in from 10 to 12 days following the uredial inoculation (Plate I, A.). The five control infections, however, around the periphery of which no urediospores were sown, remained haploid.

Uromyces Fabae

Following the method employed to secure haploid infections in the experiments just mentioned, 60 well-isolated monosporidial infections of the strain of *U. Fabae* inhabiting *Lathyrus venosus* Muhl., were secured on seedling hosts

of *L. ochroleucus* Hook. and *L. venosus*. When these infections were 16 days old, there was applied to 50 of them composite pycnial nectar drawn from infections other than those used in the experiment. Seven days after the transfer of nectar, 49 of the 50 infections receiving it produced aecia. Within three days after the appearance of aecia, 17 of the infections produced uredia in association with the aecia (Plate I, B). The infections to which no nectar had been applied remained haploid during the course of the experiments.

Thirty compound infections, each formed by the union of two monosporidial infections, were under observation concurrently with the monosporidial infections just mentioned. When these infections had completely coalesced, 17 of the 30 produced uredia. The uredia appeared in one component several days in advance of uredia in the other. No aecia developed in any of the infections, and the 13 infections that did not produce uredia remained in the haploid condition. It is improbable that diploidization of the 17 infections producing uredia was effected by means of pycniospore intervention, that is to say, by the interflow of pycnial nectar from one component to the other, for, during the winter months, pycnial nectar was sparingly produced, and what was produced usually collected in the centre of each infection.

In another experiment, urediospores were sown near the periphery of each of ten isolated monosporidial infections. Ten days or so later, when the diploid (uredial) and haploid infections had coalesced, all of the ten formerly haploid infections produced uredia and aecia in association and at the same time. Two haploid infections near the periphery of which no urediospores had been sown remained unchanged.

Perhaps in this rust the short-cycling tendency just noted is not an unusual phenomenon. It seems that, as early as 1863, de Bary (8) encountered in this rust a similar behaviour, for he states that "in some instances the same mycelium which produces aecia gives rise to a few isolated uredia".

Phragmidium speciosum

Small plants of a species of wild rose (*Rosa blanda* Ait.) were inoculated in the greenhouse with sporidia of *P. speciosum*. Altogether, 150 haploid infections were secured. In addition to these, there were 15 compound infections, each formed by the coalescence of two haploid infections.

Following the method already referred to, composite pycnial nectar was applied to 100 of the haploid infections. The other 50 were kept as a control. Ten days after the nectar was transferred, 91 of the 100 infections receiving it produced aecia. The control infections to which no nectar was applied remained unchanged. Of the compound infections, 8 of the 15 produced aecia.

Diploidization of haploid infections by means of uredial infections was not possible in this rust, as urediospores are wanting. Repeated attempts to diploidize haploid infections by sowing aeciospores of this rust near their periphery failed. The aeciospores did not infect the leaves of the host plant, although they germinated freely in hanging drops of tap water. As this rust

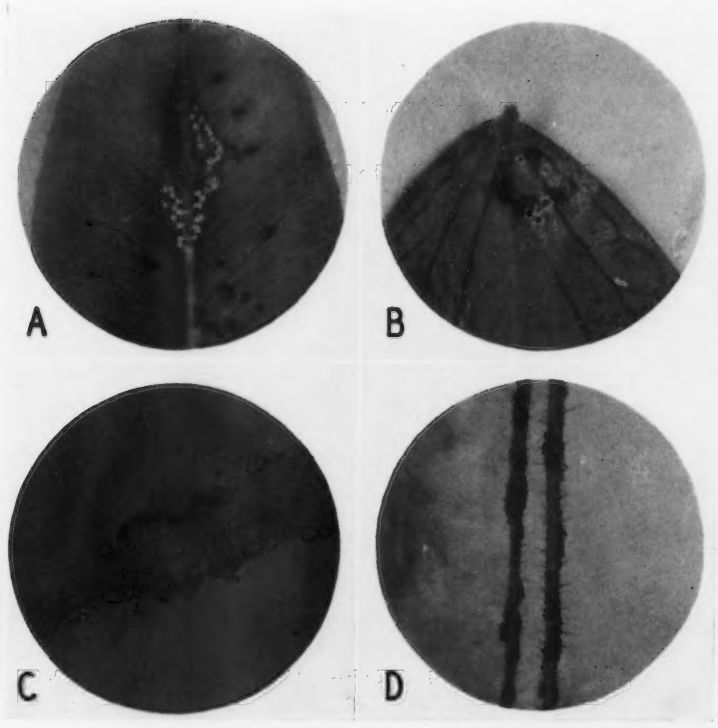

PLATE I. A. *A haploid infection of Uromyces Trifolii hybridi which has been diploidized by interaction with diploid (uredial) infections. Aecia have developed in it.* B. *A monosporidial infection of U. Fabae to which composite pycnial nectar was applied. It became diploidized and produced aecia and uredia in association.* C. *A cross section of an infection of Phragmidium speciosum on a rose leaf, showing pycnia on both the upper and lower surfaces.* × *100.* D. *Telia of P. speciosum on rose twigs. The infections earlier produced pycnia and aecia. One-half natural size.*

is autoecious, the behaviour of these aeciospores is of interest. Perhaps they might be capable of infecting some other species of *Rosa*, or a species belonging to an entirely different genus. This possibility was not investigated on account of the time of year at which the experiments were made. It should be pointed out, however, that in this rust the telia arose in the same infections that produced the aecia, and not as a result of aeciospore infection.

The pycnia of *P. speciosum* are, in general, developed equally abundantly on the upper and lower surfaces of infections on rose leaves (Plate I, C). Pycnial paraphyses are wanting, but flexuous hyphae and pycniospores are present and nectar is copious. Telia can readily be obtained on the leaves under greenhouse conditions, but under natural conditions they are found mostly on the young stems of the host (Plate I, D), for defoliation generally occurs in Western Canada before telia can fully develop in leaf infections.

When the pycnial nectar of 29 isolated monosporidial leaf infections was intermixed on the lower surface only, 26 infections produced aecia six days afterwards. Consequently, in this rust, nectar transfers, when made on the under-surface of leaf infections, are as effective in promoting aecial production as when made on the upper surface.

From the results of the experiments described above, it is concluded that *Uromyces Trifolii hybridi*, *U. Fabae* and *Phragmidium speciosum* are heterothallic, and that, in addition, *Uromyces Fabae* may shorten its life-cycle occasionally by omitting the aecial stage.

Homothallic Species

Puccinia coronata Elaeagni

The uredial and telial stages of *P. coronata Elaeagni* occur on a variety of *Calamagrostis elongata* (Kearney) Rydb.,[1] and the aecial stage occurs on *Elaeagnus commutata* Bernh.

During the winter months of 1936–7, young plants of *E. commutata* were inoculated with sporidia of *Puccinia coronata Elaeagni* by the method of inoculation previously mentioned. Each plant was but lightly inoculated, so that, as a rule, only two or three infections occurred on any plant, and usually only one infection was present on any leaf. The inoculations were not all made on the same date, but all were made during the winter months (December to March). At that time, as already mentioned, practically no insects were present in the greenhouse, but as a precautionary measure each inoculated plant when taken from the moist chamber was placed in a screen-wire cage. This precaution was taken as Allen (5) had found conidia in infections of *P. Malvacearum* and had suggested that these might be transferred by insects from one infection to another, or to the immediate vicinity of another infection, and, on germination, might enter the leaf tissue and produce

[1] *Fraser and Ledingham (15) state that the late Dr. M. O. Malte, while Dominion Agrostologist, considered the grass host of this rust to be a dry land form of Calamagrostis elongata, and for this reason that name is adopted here.*

mycelia that would interact with the mycelium of the original infection and diploidize it. There was, therefore, the possibility that, in monosporidial infections of *P. coronata Elaeagni*, similar conidia might be present and that, even in the absence of pycnia and pycniospores, diploidization of such infections might occur through the agency of insects. In this connection, it may be stated that during the course of the experiments no insects were observed on the plants.

Altogether, 51 infections were kept under observation. They were watched closely from the time of their earliest appearance, and in none of them was there any evidence that they were of bisporidial origin. Consequently, each of them was regarded as a monosporidial infection. In none of them could pycnia or pycnial nectar be detected with the aid of a strong hand-lens, yet 49 of the 51 infections produced aecia spontaneously within 36 days from the date of inoculation.

There was considerable irregularity in the age at which the different infections produced aecia. A few produced them ten days after inoculation, at which age the infections were quite small and only two or three aecia were present in each infection. In the remaining 26 days during which the infections were kept under observation, some of the infections formed aecia at one age, others at another. Ultimately as already mentioned, only two of the 51 infections failed to produce aecia, and even these two might have produced aecia had they been retained longer. The spontaneous diploidization of these infections indicates that *P. coronata Elaeagni* is homothallic.

Why some infections required a much longer time than others to produce aecia is not understood. The infections developed aecia in much the same haphazard manner as would haploid infections of *P. Helianthi* if left exposed to the occasional visit of insects; yet, in a rust like *P. Helianthi*, if no insects were present, as was the case in this experiment, most of the infections at least would have remained undiploidized. No such delay, however, occurs in the production of telia by *P. Malvacearum*, another homothallic rust, as has just been mentioned in the production of aecia in *P. coronata Elaeagni*.

Although this rust has been described as lacking pycnia (15), abortive pycnia (Plate II, A) occasionally are present. These can only be detected by sectioning the infections. They are composed entirely of hyphae, and show no evidence of pycniospores. Pycnial nectar is not produced, and paraphyses are absent. Besides, no flexuous or "surface" hyphae protruding from the infections were observed, so that it seems impossible that conidia, such as those mentioned by Allen (5) as occurring in *P. Malvacearum*, were present. There was not the possibility, therefore, of newly formed conidia causing infections near existing infections and thus affording an opportunity for the interaction of two mycelia of opposite sex. The mechanism by which diploidization can be effected by insects seems to be absent in this rust.

The sporidia of *P. coronata Elaeagni* when first abstricted are uninucleate, but shortly afterwards, even before they germinate, the majority of them

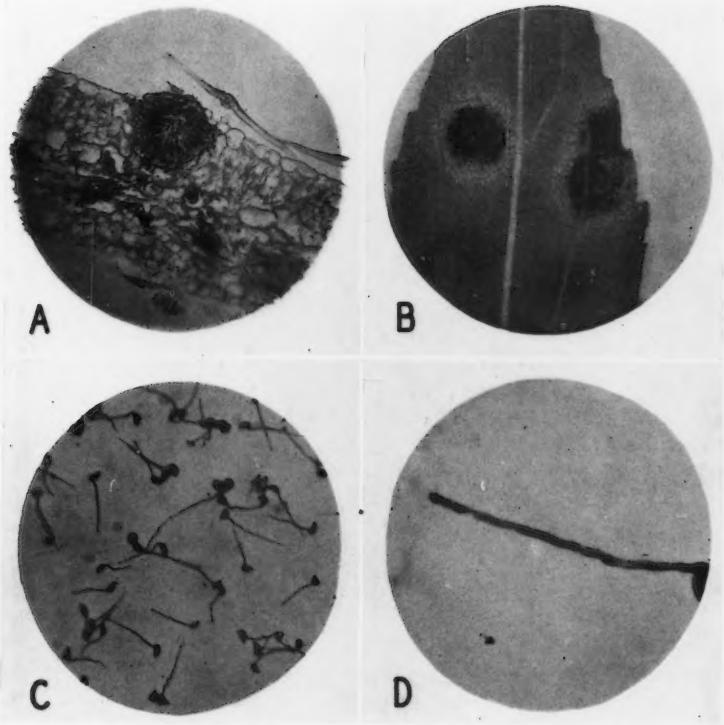

PLATE II. A. *Cross section of an infection of Puccinia coronata Elaeagni, showing an abortive pycnium.* × *100.* B. *A single and a compound infection of P. Xanthii. Both have produced telia simultaneously. About natural size.* C. *Germinating sporidia of P. Grindeliae.* × *200.* D. *A germinating sporidium of P. Grindeliae showing nuclei in various stages of division.* × *600.*

become binucleate. Occasionally when sporidia are about to germinate, some of them may contain three or even four nuclei.

As far as observed, the hyphae of young infections and their aecial primordia are uninucleate. The binucleate condition seems to appear first just prior to the differentiation of the first stalk cells of the incipient aecium, but how it is brought about was not definitely determined. As, in an examination of many sections, no cell fusions were observed, it seems reasonable to conclude that the binucleate condition arose as a result of nuclear division without cross wall-formation.

Puccinia Malvacearum, *P. Grindeliae*, and *P. Xanthii*

An investigation was also made of the sexual behaviour of *P. Malvacearum*, *P. Grindeliae*, and *P. Xanthii*. The teliospores of these rusts germinate readily without undergoing any prolonged dormancy period, so that, during the winter season of 1937–8, successive sporidial inoculations of their respective hosts were made with each of the three rusts. Shortly after the infections became visible, an examination was made with a strong hand lens to determine which infections were monosporidial and which bisporidial in origin, in order that their identity would not be confused.

One thousand and ten monosporidial infections of *P. Malvacearum* were obtained on *Althaea rosea* (L.) Cav. and *Malva rotundifolia* L.; 150 of *Puccinia Grindeliae* on *Grindelia squarrosa* (Pursh) Dunal; and 350 of *Puccinia Xanthii* on *Xanthium commune* Britt. All of these infections were well separated, and there is little doubt that all, or practically all, were monosporidial in origin. When from 10 to 12 days old, all of the infections produced telia spontaneously.

Besides the many monosporidial infections just mentioned, 650 compound infections, each formed by the coalescence of two monosporidial infections, were randomly selected from the three rusts for observation. All of these infections produced telia simultaneously in both components and within the same length of time as that required for telial production in the monosporidial infections. Both components of each compound infection produced telia spontaneously at their centres, as if each component had become binucleate independently of the other component (Plate II, B). This observation was confirmed later by an examination of microtome sections cut from several compound infections that had just produced telia. The sections showed binucleate mycelia (at the centres of the two components) separated by an intervening area of uninucleate mycelium. No hyphal fusions were detected, and there was no evidence to indicate that nuclear interchange had taken place between the components. The behaviour of these compound infections is in striking contrast to those of a heterothallic rust, such as *Puccinia Helianthi*. In the latter rust, aecia develop in about one-half of the compound infections (10, 11, 12). Moreover, if, in a compound infection having components of opposite sex, the intermixing of the pycnial nectar of the two components is prevented, one component will invariably produce aecia in advance of the other component (10). In the three rusts now under discussion, all of the compound

infections produced telia, and, without exception, produced them simultaneously in both components. From these results it is evident that these three rusts are homothallic.

In these three rusts the promycelium produces four uninucleate sporidia, which soon after abstriction germinate readily in a film of moisture (Plate II, C). Nuclear division occurs rapidly after germination takes place and two or more nuclei, in various stages of division, may be seen in the germ tube (Plate II, D). The mycelium of monosporidial infections is definitely uninucleate, but when the infections are about eight days old, the binucleate stage, as already indicated, usually appears near their centres.

From an examination of a large number of sections cut from five- to seven-day-old infections of the three rusts, it was evident that the binucleate condition regularly arises as a result of nuclear division without cross-wall formation, and not by nuclear migration. This finding seems to be in agreement with that of Ashworth (7), although she reported infrequent nuclear migrations.

The binucleate cells then become club-shaped (Plate III, A) and the two nuclei in each divide. A cross wall is then laid down, dividing the club-shaped cell into an apical and a basal part, each part containing two nuclei that fuse into one as the spore matures (Plate III, B). Nuclear irregularities, however, sometimes occur and club-shaped cells (Plate III, C) containing three or more nuclei may from time to time be encountered.

All of the rusts so far known to be heterothallic, including the three of the present investigation, possess well developed pycnia. Only four rusts are known to be homothallic, the one reported by Ashworth (*P. Malvacearum*) and the three of the present investigation. Of these four, *P. Malvacearum* and *P. Xanthii* lack pycnia entirely, *P. coronata Elaeagni* produces occasionally abortive pycnia, but generally they are absent, and *P. Grindeliae* produces pycnia only on one of its hosts (*Xylorrhiza*). It would appear from this limited evidence that the presence of pycnia in a rust would indicate that the rust is heterothallic, and that the absence or underdevelopment of pycnia in a rust would indicate that the rust is homothallic. The fact that *P. coronata Avenae* is heterothallic and possesses well developed pycnia while *P. coronata Elaeagni* is homothallic and develops occasionally abortive pycnia, would seem to support the assumption that homothallic rusts may have been at some stage in their development heterothallic. If the absence or underdevelopment of pycnia in these homothallic rusts be regarded as evidence of degeneration, the assumption seems to receive further support.

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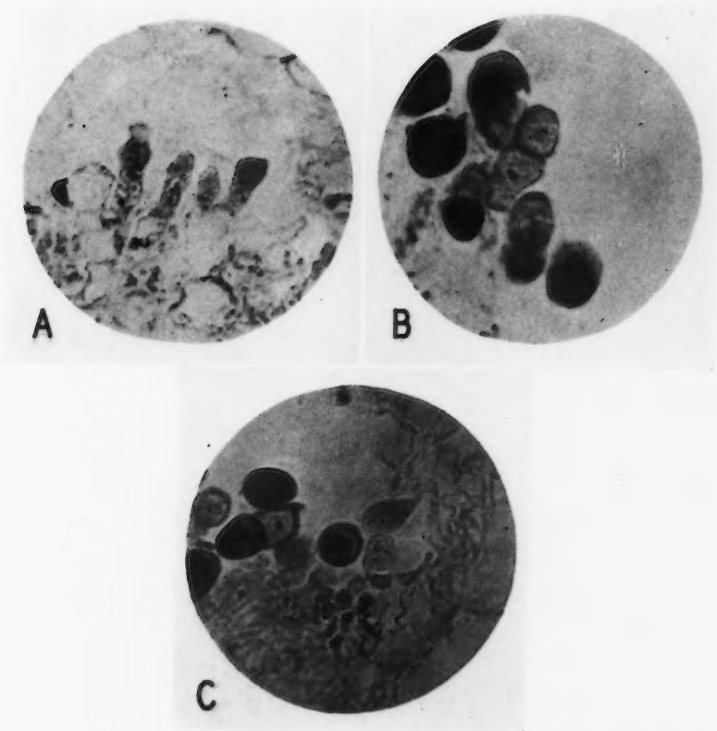

PLATE III. A. *Part of a section cut from a monosporidial infection of Puccinia Malvacearum showing four club-shaped cells. In the two cells at the left and the one at the right, one nucleus has divided and the two daughter nuclei have drawn apart; the other nucleus is still undivided. The two nuclei of the cell third from the left have not yet begun to divide.* × *350.* B. *A maturing teliospore of P. Malvacearum with the two nuclei in the lower cell in close contact.* × *600.* C. *A section of a young telium of P. Malvacearum, showing cells with one, two and three nuclei.* × *550.*

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HISTOLOGY OF SOME PHYSIOLOGICAL DISORDERS OF THE APPLE FRUIT[1]

By Mary MacArthur[2]

Abstract

The histological similarities and differences between the boron-amenable disorders, internal cork, corky core, and drought spot, and the non-boron-amenable disorder, bitter pit, are presented. Blotchy pit is discussed as a borderline type, since one end of a graded series is similar to internal cork and the other is indistinguishable from bitter pit. On the basis of histology water core is not placed in either of the two groups.

Starch retention, in localized or diffuse necrotic areas, is common to both groups of disorders. External papillations occur on the walls of cells in close proximity to the lesions. Abnormal meristematic activities coexist with boron deficiency. These are (1) a cork cambium partially or completely walling off a lesion, (2) massed linear cells, heavily papillated, and (3) reactivated individual cells or groups of cells. All three types of abnormal cells may be present in internal cork and drought spot, but no cork cambium occurs in corky core.

The currently used diagnostic characters of the physiological disorders of the apple are based almost exclusively on the gross morphology of the lesions. The present study was undertaken in an attempt to differentiate the various disorders from a histological point of view. It is realized that an adequate inquiry into the problem should comprise a detailed study of the fruit throughout the season for the purpose of correlating growth with initiation and progress of histological changes. While this study is preliminary, sufficient progress has been made on general anatomical characters to justify its presentation.

Methods

Collections were made of six disorders in 15 varieties of apples in orchards of Ontario, Quebec, and Nova Scotia. More than 50 collections were made during the late summer and autumn of 1938.

Blocks of affected apple tissue up to 1 cc. in volume were killed and fixed in formalin-acetic-alcohol. The samples were dehydrated in an *n*-butyl alcohol series and embedded in paraffin, m.p. 53 to 55° C. Sections 10 to 15μ thick were made, and various stains, such as Haidenhain's iron alum hematoxylin, crystal violet and orange G, safranin and fast green, were used. In addition, the presence of suberized tissue was tested with Sudan IV, the sections being mounted in glycerin jelly to avoid the de-staining concomitant with the use of xylol-balsam. The presence of pectin substances was investigated by the use of Ruthenium red and by the Prussian blue precipitate formed on treatment of such substances with ferrous chloride, potassium ferrocyanide and hydrochloric acid.

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The disorders examined were Internal cork, Corky core, Drought spot (Superficial cork), Bitter pit (Tree pit, normal type), Blotchy pit (Tree pit, blotchy type) and Water core.

Observations

Internal Cork

The three varieties—Fameuse, Ontario, and Delicious—from which samples were taken all showed varying degrees of the typical pebbly surface associated with severe cases of the disorder. A second diagnostic character of internal cork is the greenish tissue delimiting a lesion. Frequently this greenish tinge is absent, particularly in later-formed cork. Such lesions then have the gross appearance of bitter pit. Both types of lesions were examined.

Whether or not the peripheral coloration of the lesion is present, definite lesions of internal cork exhibit microscopically either a more or less continuous delimiting cambium with its derived tissues (Fig. 1), or a closely compacted growth of abnormal linear tissue (see following). Mix (6) figures a ladder-like arrangement of nearly rectangular cells around the "corky" portion. He states that these are healthy cortex cells. Tests with Sudan IV indicate that the cambium is a cork cambium. In the suberized layer, the phellem is more extensive than the phelloderm. The designation "cork" in this disorder is not a misnomer. The lesion was originally termed "cork" because the brown spongy, internal necrotic area superficially resembles cork. However, there is no suberized tissue in this region.

External to the phellem are numerous "reactivated" cells. The walls of such cells are definitely thinner and the nuclei are prominent. Interspersed among the reactivated cells, and often filling entirely the intercellular spaces, is a second type of abnormal cell. This abnormal tissue (Fig. 2) is composed of attenuated cells. The writer has observed numerous cases of the initiation of such tissue by *(a)* division of a cortical cell, and *(b)*, the linear growth of phellem cells. In *(a)*, a daughter cell from the division projects into the intercellular space and linear growth from subsequent divisions is continued at the distal end. Branching occurs frequently. Linear growth from the outer phellem cells is similar. The tissue resembles the transversely septate hyphae of the Ascomycetes, but the cells are many times as large, and are heavily papillated. Although this tissue is not discussed in the text, it is pictured by Verner (12, Plate 5B). In this plate, it appears as if the abnormal tissue arises in close relation to the cork cambium, which cuts off the hypodermal tissue in cracking of apples. It is similar to the tissue extending into the locules in woolly core of apples (10). Many of the apples examined in this study were affected with woolly core, but there was no correlation between the presence of such tissue arising from the split cartilaginous endocarp and abnormal linear cells in other tissue.

Both abnormal types of apple cells contain starch grains which are significantly smaller than the starch grains of normal cells. These smaller starch

grains do not stain as deeply with either crystal violet or iodine-potassium-iodide.

The walls of normal-appearing cells contiguous to the lesions are also papillated. The prominence of the papillations decreases with increasing distance from the lesion. The papillae may range from slight extensions of the wall to finger-like or mushroom-shaped protrusions. It is believed that they are concentrations of the middle lamellae. They are always external and stain conspicuously with hematoxylin.

The necrotic area of the internal cork lesion is composed mainly of collapsed cells with imprisoned starch grains. Closely but unevenly applied to the inner surface of the generally ruptured cell is a brownish, gummy or mucilaginous substance. Micro-chemical tests demonstrate that the mass is of a pectic nature. However, it is different from that found in the walls, since the walls of cells in the necrotic area stained with Prussian blue, which gradually faded to a paler blue, while the Prussian blue stain of the mucilaginous masses slowly changed to a green, and finally to a yellow-green.

Vascular bundles which penetrate or approximate the lesion are affected. Plugging in the form of a finely-granular substance appears in the phloem cells. In addition, a second disorder may be present in the same tissue. This is in the form of masses that are yellow in unstained section and that stain deeply with hematoxylin. These masses are found between normal phloem cells. This may indicate gummosis of part of the phloem, or formation of intercellular spaces by splitting of the middle lamellae and subsequent secretion of pectic substances, which finally fill the space. It is somewhat similar to the disorder found in the phloem of the potato affected with leaf-roll (1). In many cases the xylem of the bundle is also affected. The secondary thickening of the spiral tracheids is coated with an undetermined substance which prevents the clear staining reaction effected with normal cells.

Corky Core

The varieties McIntosh Red, Scarlet Pippin, Ben Davis, Baldwin, and Northern Spy were affected with this disorder. The gross morphological character of the disorder is a slight browning in the pith area, usually in the regions to the right and left of the dorsal carpel bundle and, in more severe cases, browning of the whole pith (core region) centripetally from the main fibrovascular bundles. The more severely affected fruits may have lysigenous cavities formed by the collapse and separation of large groups of cells.

Microscopically the lesion is diffuse. The cells are more or less tangentially collapsed. Starch grains are imprisoned between the approximated cell walls. Starch may be completely absent from the surrounding tissue, except in those younger parenchyma cells that are added to the ground tissue by the cambial activity of the larger fibrovascular bundles. When reactivated cells are present, they are cuboidal in shape. A long, narrow pith cell which is stimulated divides several times. Thin cross walls are formed, and the final result is a pith cell divided into a linear series of cuboidals. Abnormal linear growth similar to that found in internal cork is always present, but is some-

what diffuse (Fig. 3) and may be sparse. This abnormal tissue is found in the intercellular spaces contiguous to the necrotic area. It may enter the lacunae formed on the collapse of cell groups. As with internal cork, the walls of the affected cells stain more deeply. Papillae are present, but are not as conspicuous as those accompanying internal cork. Small quantities of a gummy material similar to that in the previous disorder is closely applied to the inner side of the ruptured or collapsed cell wall.

Drought Spot or Superficial Cork

This disorder was examined in the varieties McIntosh Red and Ben Davis. The gross diagnostic character of the disorder is a rugosity of certain localized areas. Cracks may develop and the fruit may become malformed. Later drought spot may have a russetted appearance or simply a cuticular checkering. However, in Ben Davis definite lesions are visible through the epidermis. Such lesions may or may not involve the surface. In all cases, the outer tissues primarily are affected.

As normal growth of the apple proceeds, the epidermal and hypodermal cells become tangentially elongated (11). If these cells are affected early, they are rounded, have a heavily cutinized epidermis and a thick-walled hypodermis. The rounded hypodermal cells are often loosely arranged, since they are radially disrupted by the tangential torsion and the radial pressure of the subjacent growing tissue. In light cases of the disorder, the hypodermal cells are severely flattened but not separated. The lumen is a narrow slit filled with a dark-staining, finely granular deposit. Since the outer tissues in drought spot areas have little extensibility, the continued growth of the underlying tissues causes cracking. It is in these more severe cases that a cork cambium is initiated in the outer cortex (Fig. 4). Epidermis and hypodermis are then sloughed off irregularly. Some of the cortical cells collapse and imprison starch grains. Throughout the intercellular spaces and centripetally from the outer limiting periderm layer, abnormal linear tissue is interspersed. In the hypodermal area, the papillae of the cell walls may bridge the narrow intercellular spaces and so give the appearance of a lattice.

In Ben Davis the lesion closely approaches internal cork in appearance (Fig. 5). The cambial layer is less continuous but the abnormal tissue is much more extensive. Reactivated cells of both cuboidal and filler type are present. The central region of the lesion is composed of radially collapsed cells with imprisoned and generally clumped starch grains. Such grains are embedded in a matrix probably composed of breakdown products of the protoplasm. Serial sectioning discloses that a fibrovascular bundle of the cortex is in contact with a lesion. Some of the sieve tubes and parenchyma of the bundle show collapse. Often the bundle cells are forced apart by abnormal linear growth. In addition, the bundle cambium may initiate cuboidal parenchyma. Papillae occur on the cell walls and are more conspicuous in close proximity to the lesion.

Bitter Pit (Tree pit, normal type)

Bitter pit was examined in Cooper's Market, Stark, Blenheim, Red St. Lawrence, Baldwin, and Northern Spy. The surface lesions of these samples were uniformly coloured, red, brown or green, were up to 7 mm. in diameter, slightly to not at all depressed, and involved tissues external to the main fibrovascular bundles. In some of the samples, other disorders such as corky core or water core were present.

When starch grains are equally distributed throughout the external tissues and when there is yet no breakdown, cell walls in certain areas stain intensely with the Prussian blue precipitate in tests for pectin substances. Since the necrotic areas of typical bitter pit exhibit a similar but stronger staining reaction, it is believed that the above reaction indicates an early stage of the disorder. These same areas also stain heavily with Ruthenium red. Later, a few cells collapse. With further development collapsed cell walls withdraw from centralized areas, leaving small to larger lacunae, and starch grains are held between collapsed walls (Fig. 6). The starch is usually embedded in an amorphous substance which is probably the dead and discoloured protoplasmic contents of the affected cells. At this stage there may be more starch in the surrounding healthy cells. Still later, with starch hydrolysis accompanying the maturation of the cells, starch disappears from the healthy tissue and the starch content of the necrotic areas becomes conspicuous.

These observations on the starch are believed to be evidence that normal starch metabolism is disrupted early, and that the presence of starch grains in the necrotic areas alone at later stages is merely incidental, since by their location in affected areas they were outside the sphere of normal metabolic, and therefore hydrolytic, activity. This is in accordance with the views of Smock (8) concerning the persistence of starch in bitter pit, and again, "starch persistence may be concomitant with the trouble rather than causal." (9).

In most cases of bitter pit examined, serial sections revealed that the lesion was in contact at some point with a fibrovascular bundle (7, 9). Only when the bundle passed directly through a lesion was definite disorganization of the bundle evident. In transverse sections of bundles at the periphery of lesions, affection of the sieve tubes and parenchyma similar to the collapse observed in drought spot was noted. It is impossible without the investigation of earlier collections to determine whether this disturbance is of primary importance or is to be considered an effect of the disorder.

Neither abnormal linear growth, reactivated cells, nor a secondary cambium formation was observed in any sample of bitter pit when bitter pit alone was present. The cell walls were papillated and, as with the disorders previously described, the papillae were well defined near the lesions.

Blotchy Pit (Tree pit, blotchy type)

Blenheim, Stark, Delicious, Tolman Sweet, and Deacon Jones affected with this disorder were examined. Macroscopically the lesions approximate bitter pit in appearance, but are larger and are dark green mottled with brown.

Anatomically, there are two extreme types of blotchy pit. Type I (Fig. 7) closely resembles internal cork, and Type II (Fig. 8) is identical in appearance with bitter pit. Between these two extreme types gradations are found. In Type I a discontinuous cambium defines the lesion. At the periphery of the lesion where cambial activity is not present, the intercellular spaces and lacunae of collapsed cell groups are packed with abnormal linear tissue. Reactivated cells, mainly of the filler type (Fig. 2) are also present. In Type II there is neither cambial activity nor abnormal growth.

In the necrotic areas of both types there is cell collapse, lacunar formation and retention of starch grains. The dead, coagulated protoplasm adheres to the walls. The walls of living cells are always papillated.

In the intergrade types, a cork cambium cuts off external cork in a shallow hypodermal lesion, or, when cork is not present, the sub-epidermal cells are radially flattened, tangentially elongated and thick-walled. The narrow lumen is filled with a dark-staining material. Some few abnormal linear cells are found in the intercellular spaces of the outer cortex.

Water Core

Blenheim, Red St. Lawrence, Northwestern Greening, Delicious, and Tolman Sweet were affected with water core. Watery injection of the tissues is the diagnostic character. When the evidence is external, the skin is darker in colour, the injected areas being seen through the epidermis.

When the disorder was located in the core region, the primary fibrovascular bundles were involved. Cortical vasculars arising from the affected primaries exhibit a water-logged sheath (Fig. 9). Large water-core areas may finally show a breakdown of the soggy type (Fig. 10).

Microscopically, no abnormal growth occurs when water-core alone is present. A few weakly-staining starch grains are found, but most of the starch has become hydrolyzed. The middle lamellae stain lightly with hematoxylin, evidence that the cell wall pectins are changed. Of the bundles, only the parenchyma cells of the sheaths are affected. These are shrunken and collapsed. The cell walls are not papillated.

For convenience of comparison, the anatomical characters of the disorders investigated are arranged in a table.

Discussion

The physiological disorders of the apple fruit that occur on the tree are of two classes, boron-amenable and non-boron-amenable. Of the disorders examined in this study, internal cork, corky core, and drought spot are alleviated by the use of boron in the nutrition of the tree, but bitter pit is not affected. Most of the nutritional work done on blotchy pit indicates that it is a form of bitter pit, but Carne and Martin (2), in their boron treatments of Cleopatra, state that this variety apparently responded to liquid injections of boric acid. However, they doubted the significance of the results in view of the highly significant difference in fruit size. Many other experiments of

TABLE I

A SUMMARY OF THE ANATOMICAL INVESTIGATION OF SOME DISORDERS OF APPLES

Disorder	Variety	Cell collapse	Starch retention	Papillae	Abnormal growth		Cambial activity
					Linear	Reacti-vated	
Internal cork	Fameuse	+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	+
	Ontario	+	+	+	+	+	+
		+	+	+	+	+	+
	Delicious	+	+	+	+	+	+
Corky core	McIntosh	+	+	+	+	+	−
		+	+	+	+	+	−
		+	+	+	+	−	−
		+	+	+	+	−	−
		+	+	+	+	−	−
	Ben Davis	+	+	+	+	+	−
	Baldwin	+	+	+	+	−	−
		+	+	+	+	−	−
	Northern Spy	+	+	+	+	−	−
	Scarlet Pippin	+	+	+	+	−	−
Drought spot	Ben Davis	+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	−	−
		+	+	+	+	−	−
	McIntosh	+	+	+	+	+	−
		+	+	+	+	−	−
		+	+	+	+	−	−
		+	+	+	+	−	−
Bitter pit	Cooper's Market	+	+	+	−	−	−
		+	+	+	−	−	−
	Baldwin	+	+	+	−	−	−
		+	+	+	−	−	−
	Blenheim	+	+	+	−	−	−
	Northern Spy	+	+	+	−	−	−
		+	+	+	−	−	−
	Red St. Lawrence	+	+	+	−	−	−
	Stark	+	+	+	−	−	−
		+	+	+	−	−	−
Blotchy pit	Blenheim	+	+	+	+	+	+
		+	+	+	+	−	−
	Stark	+	+	+	+	+	+
		+	+	+	+	+	−
		+	+	+	+	+	−
		+	+	+	+	−	−
		+	+	+	−	−	−
	Delicious	+	+	+	+	+	−
	Tolman Sweet	+	+	+	+	+	−
		+	+	+	−	−	−
		+	+	+	−	−	−
	Deacon Jones	+	+	+	−	−	−
Water core	Blenheim	−	−	−	−	−	−
	Delicious	−	−	−	−	−	−
	N. Greening	−	−	−	−	−	−
	Red St. Lawrence	−	−	−	−	−	−
	Tolman Sweet	−	−	−	−	−	−

the same nature are inconclusive because of the absence of the disorder on both treated and control trees.

Internal cork, corky core, and drought spot all showed abnormal growth. None was found in bitter pit, while blotchy pit was intermediate. One type of lesion is similar to bitter pit, others approach the various boron-amenable lesions. The difficulty of obtaining definite results in blotchy pit may be due, in part at least, to this difference in types.

Living tissues in their metabolic activity pass through three phases. Fig. 11 illustrates the normal cycle of activity. It is known that internal cork, drought spot, and possibly corky core appear earlier in the developmental history of the fruit than bitter pit. It is suggested that the first three disorders appear in phase 1, when anabolic activity is predominant; that blotchy pit of the several types appears in phases 1, 2 or 3, and that bitter pit appears only in 3.

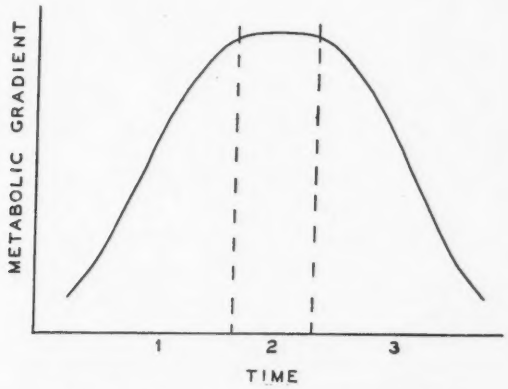

FIG. 11. *Theoretical curve of metabolic activity.*

The majority of the tissues of the fruit are fundamental in nature, and are capable of stimulation to meristematic activity, provided that the cells are in the anabolic stage of metabolism. Stimuli may be varied to call forth such a reaction. Intumescences of cabbage which show cambial activity similar to that found in internal cork, drought spot and certain blotchy pit lesions may be caused by a condition common to exposure to acid or alkaline vapours, to freezing, to drying by excessive transpiration from exposed areas, to aphis punctures, to breaking the leaf surface or to injections of water that loosen the epidermal layer (5). Haberlandt (4) attributes a similar growth in storage cells of the potato tuber to a growth-producing enzyme in the companion cells of the phloem. Gilbert (3) found a cork cambium surrounding the necrotic areas of potato tubers affected with net necrosis. He believed the meristematic activity was due to an accumulation of soluble nutrients at the blocked walls.

Therefore, all such abnormal meristematic activity is initiated by some stimulus. It is conceivable that deficiency of boron initiates a physiological change in localized areas. The death of the cells of such areas and the meristematic activity of contiguous cells in the anabolic phase are links in the chain of reactions set up by the disrupted metabolism. In bitter pit, the factors concerned with the initiation of necrosis differ, for boron applications do not reduce the incidence of the disorder. Meristematic activity is not an adjunct of lesion formation in bitter pit. Therefore, the metabolic stage of the tissue is a factor which must be considered. There is a possibility that boron deficiency is the stimulus in some types of blotchy pit.

Starch retention in greater or lesser quantity in all necrotic areas indicates changed localized metabolism. Ergastic inclusions as starch grains are thus thrown outside the sphere of normal hydrolytic activity. The size differences between such starch grains and those of abnormal tissue are probably due mainly to the relative physiological adjustment between size of cell and size of starch grain.

It is difficult to determine whether the partial disorganization of the bundles in contact with lesions is primary or secondary. All lesions penetrated by fungus or caused by insects contained numerous globular masses of an undetermined substance. Such masses were conspicuous in the parenchyma of the proximal bundles.

The study of the disorders occurring on the tree and their relation to the storage disorders is being continued.

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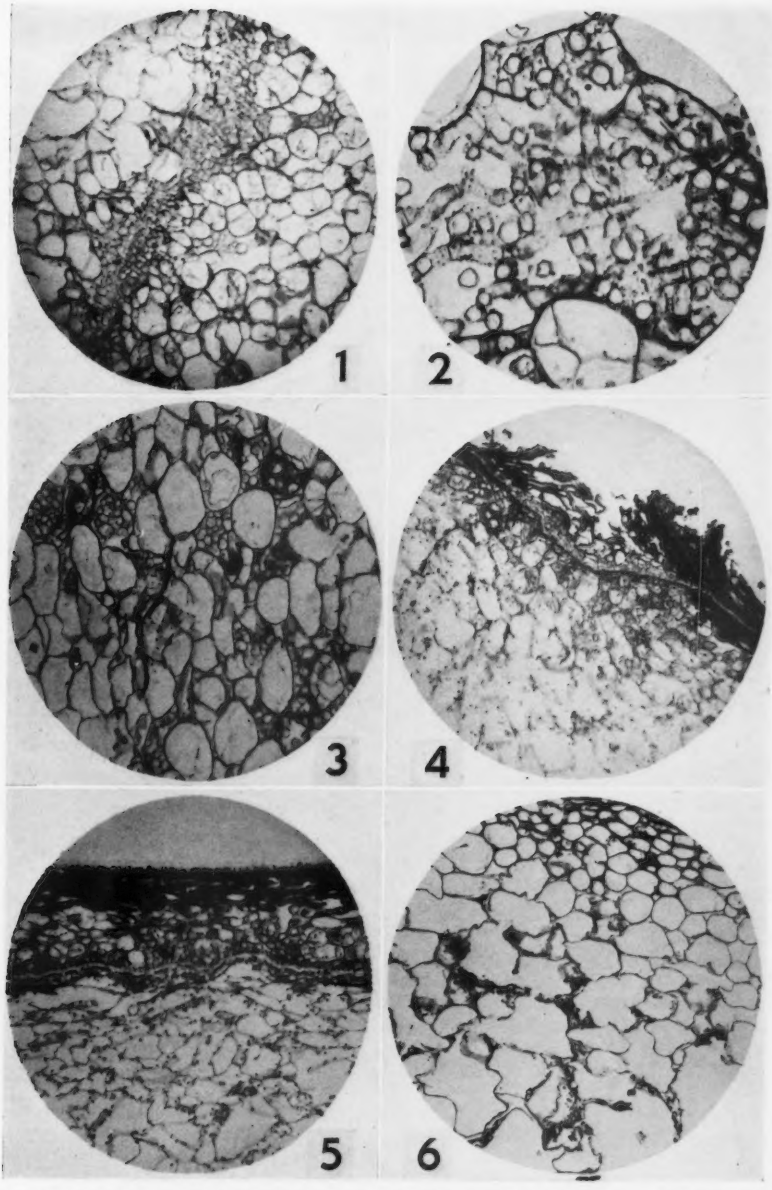
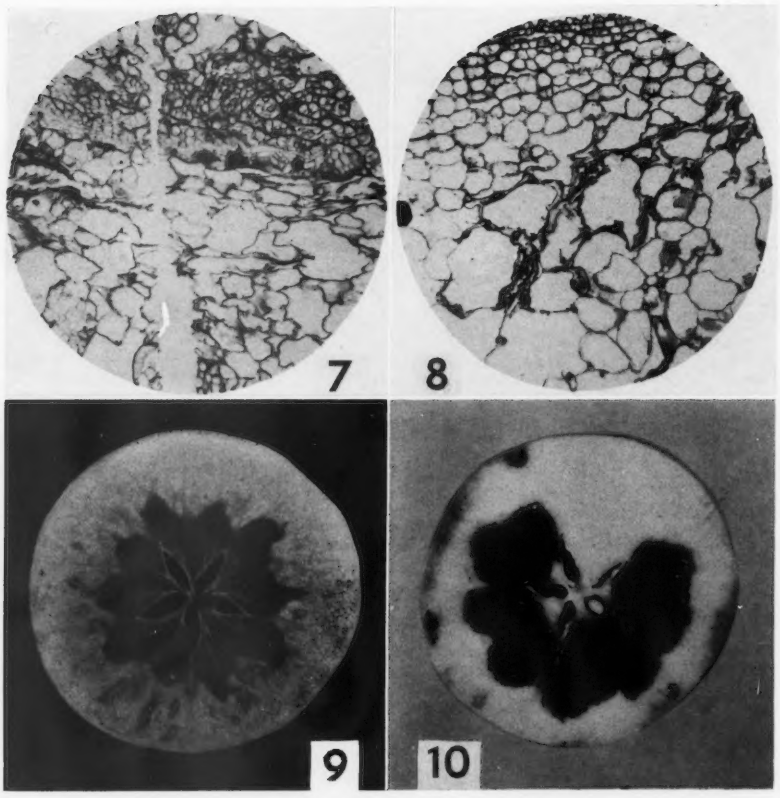


FIGS. 1 – 6. FIG. 1. *Internal cork, Ontario. Left, necrotic tissue; centre, cork cambium; right, "reactivated" and normal cells, interspersed with abnormal linear tissue.* × *80.* FIG. 2. *Abnormal linear cells with conspicuous papillae. Reactivated cell of "filler" type at bottom of figure.* × *215.* FIG. 3. *Corky core, Northern Spy.* × *80.* FIG. 4. *Drought spot, McIntosh Red. Cambium in outer cortex. The scattered dark spots are starch grains.* × *80.* FIG. 5. *Drought spot, Ben Davis, showing cork cambium. Necrotic tissue in lower part of figure.* × *80.* FIG. 6. *Bitter pit, Baldwin. Note retention of starch and papillated walls. Compare with Fig. 8.* × *80.*



FIGS. 7–10. FIG. 7. *Blotchy pit, type 1. Top, abnormal linear cells; upper centre, cambium; lower, necrotic tissue. Compare with Fig. 1.* × *80.* FIG. 8. *Blotchy pit,t ype 2. Deacon Jones. Note similarity to bitter pit, Fig. 6.* × *80.* FIG. 9. *Water core, Red St. Lawrence.* FIG. 10. *Water core breakdown, soft type. Tolman Sweet.*

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STUDIES ON CERCARIAL DERMATITIS AND THE TREMATODE FAMILY SCHISTOSOMATIDAE IN MANITOBA[1]

BY J. A. MCLEOD[2]

Abstract

A biological investigation of cercarial dermatitis in Manitoba has revealed that three species of apharyngeal brevifurcate cercariae are responsible for the disease. An ecological study of the organisms and their molluscan hosts has been carried out, and descriptions of the general types of habitats are given in addition to the distribution of the organisms. A synopsis of the dermatitis-provoking cercariae and the adult schistosomes of the world is given, and the classification of the adults is revised in part. The results of a survey of mammals and birds for adult schistosomes and also the results of experimental exposures of animals are included. A new species of apharyngeal brevifurcate cercaria and two new species of *Ornithobilharzia* are described. The description of *Cercaria wardlei* McLeod, 1934, is revised.

Introduction

An investigation of the etiology of swimmer's itch was undertaken in Manitoba in 1933, and the work has been carried on periodically since that time. Prior to the beginning of the work reported for the first time herein, it had been found that this local transitory skin disease of man was identical with the so-called cercarial or schistosome dermatitis in parts of the United States and various other countries. It had also been found that the disease in this province is provoked by the penetration of the skin by one or more species of brevifurcate larval trematodes or cercariae; that the organisms and their snail hosts are abundant in Clear Lake; that three species of adult schistosomes occur locally in wild ducks and a fourth species occurred in Herring Gulls collected near Yarmouth, Nova Scotia; also that a number of cercariae had failed to penetrate into, or reach maturity in, several species of experimental mammals and birds.

To elucidate the biology of the organisms concerned and bring our understanding of them to a point where control measures of some value might be suggested, the work has been continued and expanded. The purpose of the problem reported on herein, therefore, has been:—

1. A survey of southern Manitoba for dermatitis-provoking cercariae and their molluscan hosts.

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2. A brief study of the ecological factors responsible for their abundance and distribution.
3. A continuation of the survey of birds and mammals in an effort to discover what adult schistosomes occur in this area.
4. Exposure of experimental mammals and birds to the cercariae in an attempt to complete the life-history stages.
5. A study of related but non-dermatitis-provoking cercariae.
6. A study of the literature on larval and adult schistosomes of the world to determine the relationships of the local forms.
7. A revision of the classification of the Schistosomatidae.

Historical

The phylogeny of the schistosomes is not clearly understood at the present time, but the parasitic habit of life among them is apparently one of long standing. That the adults of one species have been the inhabitants of the blood vessels of man for at least 30 centuries has been proved beyond doubt by Ruffer (129). The first species of schistosome was discovered by Rudolphi in 1819 and was of the so-called non-human type, being a parasite of birds. *Schistosoma haematobium*, the second species recorded, was a true parasite of man and was discovered by Bilharz in Egypt in 1852. It was first recorded as *Distoma haematobium* but received its present name from Weinland in 1858. The second schistosome of man, *S. japonicum*, was described by Katsurada in 1904, and a third, *S. mansoni*, also well known at the present time, by Sambon in 1907.

In the interval between 1819 and 1912 thirteen species of schistosomes were recorded, but at that time nothing was known regarding the life-histories of the organisms or their method of gaining entrance to the host. It may be noted, however, that in 1896 Looss suggested it might be by active penetration of the skin.

The species recorded during this interval and the type of host in which each was found are as follows:—

Ornithobilharzia canaliculata (Rudolphi, 1819) Odhner, 1912, from birds.
Schistosoma haematobium (Bilharz, 1852) Weinland, 1858, from man.
Schistosoma bovis (Sonsino, 1876) Blanchard, 1895, from mammals and man (?).
Bilharziella polonica (Kowalewski, 1895) Looss, 1899, from birds.
Ornithobilharzia kowalewskii (Parona and Ariola, 1896) Odhner, 1912, from birds.
Dendritobilharzia pulverulenta (Braun, 1901) Skrjabin, 1924, from birds.
Schistosoma japonicum Katsurada, 1904 (S. cattoi Blanchard, 1905), from man.
Schistosoma indicum Montgomery, 1906, from mammals.
Schistosoma spindalis Montgomery, 1906, from mammals, rarely man.
Ornithobilharzia bomfordi (Montgomery, 1906) Price, 1929, from mammals.
Schistosoma mansoni Sambon, 1907, from man.

Gigantobilharzia acotylea Odhner, 1910, from birds.
Ornithobilharzia intermedia Odhner, 1910, from birds.

In the years 1913–14, Miyairi and Suzuki demonstrated that in the case of *S. japonicum* a parthenogenetic stage is passed through in a molluscan host and that the cercarial larvae so produced gain entrance to the appropriate warm-blooded host by active penetration of the body surface. This cleared the way for further work and the chronology of events from then up to the present time is as follows:—

Schistosoma japonicum, life-history by Miyairi and Suzuki, 1913.
Ornithobilharzia turkestanicum (Skrjabin, 1913) Price, 1929, from birds.
Schistosoma haematobium and *S. mansoni*, life-histories by Leiper, 1915.
Austrobilharzia terrigalensis Johnston, 1917, from birds.
Schistosoma spindalis, life-history by Soparkar, 1918.
Trichobilharzia ocellata (Skrjabin and Zakharow, 1920) Brumpt, 1931, from birds.
Schistosomatium pathlocopticum Tanabe, 1923, from mammals, life-history by Tanabe, 1923.
Macrobilharzia macrobilharzia Travassos, 1923 (Price, 1929, 1931), from birds.
Ornithobilharzia odhneri Faust, 1924, from birds.
Dendritobilharzia loossi Skrjabin, 1924, from birds.
Schistosoma incognitum Chandler, 1926, from man.
Schistosoma bovis, life-history (?) by Faust, 1926.
Pseudobilharziella yokogawai (Oiso, 1927) Ejsmont, 1931, from birds. Life-history by Oiso, 1927.
Ornithobilharzia, 2 sp. Linton, 1928, from birds.
Schistosoma faradjei Walkiers, 1928, from man (?).
Schistosoma mattheii Le Roux, 1929, from mammals.
Macrobilharzia anhingae (Price, 1929) Price, 1931, from birds.
Heterobilharzia americana Price, 1929, from birds.
Microbilharzia chapini Price, 1929, from birds.
Pseudobilharziella kowalewskii Ejsmont, 1929, from birds.
Schistosomatium douthitti H. F. Price, 1929, from mammals.
Ornithobilharzia pricei Wetzel, 1930, from birds.
Schistosomatium douthitti, life-history by Price, 1931.
Schistosoma rodhaini Brumpt, 1931, from mammals.
Schistosoma cucassoni Brumpt, 1931, from mammals.
Trichobilharzia ocellata, life-history by Brumpt, 1931.
Bilharziella polonica, life-history by Brumpt, 1931.
Bilharziella margebowiei Le Roux, 1933, from mammals.
Schistosoma nasalis Rao, 1933, from mammals.
Schistosoma suis Rao, 1933, from mammals.
Schistosoma intercalatum Fischer, 1934, from mammals and man.

Microbilharzia manitobensis McLeod, 1936, from birds.
Microbilharzia canadensis McLeod, 1936, from birds.
Microbilharzia lari (Syn. *Ornithobilharzia lari*) McLeod, 1937, from birds.
Pseudobilharziella querquedulae McLeod, 1937, from birds.
Ornithobilharzia sp. Young, 1937, from birds.

Exclusive of the two species reported for the first time in this thesis, this makes a total of 25 species known to infest birds. Of these only three life-histories have been completed. There are also 18 species from man or other mammals, of which seven life-histories are known.

Fourteen of the above 43 species were first recorded from North America and an additional one, *Bilharziella polonica*, originally described from Poland, has since been found on this continent by Price (114). Creech and Miller (45) encountered cases of nasal granuloma in cattle in Louisiana, U.S.A., with symptoms identical to those occurring in India; but the causal organism, later described by Rao (124) as *S. nasalis*, was not recovered in America. The life-histories of *Schistosomatium pathlocopticum* and *Schistosomatium douthitti*, both originally described from America, are known. They spend their adult stages in rats and mice, Tanabe (149), H. F. Price (116). *S. douthitti* has also been found in wild muskrats *(Ondatra zibethica)* in Michigan and Minnesota by Penner (117).

The three common species of human schistosomes, undoubtedly, have been introduced many times into North America, but that they never have become established seems to be fairly certain. This may be due to existing sanitary conditions, absence of suitable molluscan hosts, or unfavourable climatic conditions.

Cercarial Dermatitis

In many countries including Canada and the United States, where native schistosomiasis is unknown or endemic, the occurrence of a transitory skin disease, popularly known as "swimmer's itch" or "cercarial dermatitis", has been known for many years. A true causal organism was first associated with it by Cort (36, 37) in Michigan, and the condition is now widely recognized to be the result of attacks by schistosome larvae. For some time the disease was thought to be confined to man, but a case in dogs has been reported recently by Herber (59). In addition, the writer has information to the effect that in earlier times on the western prairies, cattle wading in sloughs were frequently afflicted with a dermatitis on the legs and belly.*

Following the report of Cort's discovery, numerous authentic cases of cercarial dermatitis, that previously would have been attributed, no doubt, to attacks of chigoes, chiggers, larval hookworms, various insects, or put down to unknown causes, were reported. Christensen and Greene (26) reported cases from lakes in the vicinity of Minneapolis, Minnesota. Here the causal organism was found to be a schistosome larva whose adult stage is, even yet, unknown and which had been tentatively named *Cercaria elvae* by Miller (96, 97).

**Personal conversation with Mr. Stuart Criddle, Treesbank, Man.*

Matheson (88) reported an outbreak among bathers at an artificial lake at Cardiff, Wales. He considered a species of furcocercous cercaria to be the causal organism and identified it as *C. elvae*. Taylor and Baylis (151) examined similar material from the same lake a short time later and were able to produce a dermatitis in experimental subjects but identified the organism as *C. ocellata* La Val. St. George, 1855.

Szidat (147) reported a large number of cases from near Rossitten in East Prussia, in which he suspected the larva of *Bilharziella polonica;* but many experimental exposures of susceptible individuals failed to prove that this organism was a dermatitis provoker or could penetrate human skin. Vogel (153–155) observed a good many natural infections of what appeared to be cercarial dermatitis in bathers in the Holstein Lake region south of Kiel, Germany. He was successful in producing experimental infections in volunteers with *C. ocellata* from snails in the lakes where natural infections were known to have occurred. He made a careful study of the similarity of cercarial dermatitis in Germany and the skin eruptions in human beings reported by Nageli (109) from the Bodensee region in Switzerland.

It must be noted that none of these observers succeeded in identifying the adult phases of the parasites concerned. The work of Brumpt (17–19) in France, on material collected near Paris, not only confirmed the belief that *C. ocellata* was a dermatitis provoker but also that it was the larva of *Trichobilharzia ocellata* (Skrjabin and Zakharow, 1920) Brumpt, 1931. Price in 1931 (115) completed the life-history of *C. douthitti* Cort, 1916, which has since been found to provoke a dermatitis.

The etiology and epidemiology of cercarial dermatitis have been summarized in a number of articles the most valuable of which are by the following investigators: Fülleborn (58), Szidat and Wigand (148), La Rue (70), and Cort (38, 39). The disease was first reported from Canada by the writer in 1934 (92) and at that time it was thought to be confined to Clear Lake, Manitoba. However, information gathered personally during the past five years indicates that the causal organisms and their molluscan hosts are common in the majority of prairie lakes and sloughs in southern Manitoba (Fig. 2).

Dermatitis-provoking Cercariae

The number of species of larval trematodes that definitely bear the potentiality for provoking human dermatitis is uncertain at the present time. This is to be understood in view of the difficulty encountered in identifying species even by experienced workers. The larvae show very indefinite physiological characters and the tissues exhibit little differentiation. Cercariae from a single snail host show a fairly wide variation in size so that metromorphic characters are not to be relied upon entirely. No doubt, what are considered now as single species in a good many cases will be found later to represent a complex of several species.

Human susceptibility to the penetration of the organisms must also be taken into account. Apparently certain species of cercariae cannot penetrate the skins of all people and probably only a few species of those that do penetrate provoke a sensible skin reaction. This is particularly true of the schistosomes of man, concerning whose dermatitis-provoking powers there is a division of opinion.

A number of investigators have produced evidence both for and against the hypothesis that the cercariae of *S. haematobium*, *S. mansoni*, and *S. japonicum* are responsible for a dermatitis in man. A number of Japanese workers are of the opinion that "kabure", which resembles cercarial dermatitis in the United States, results from the attacks of the cercariae of *S. japonicum*. Miyagawa (100), however, maintains that this is not so, as "kabure" occurs in regions where schistosomiasis is not present and also that in endemic areas "kabure" does not result in infestations with adult *S. japonicum*. Faust and Meleney (55) state that a skin eruption does not occur among rice farmers in China where schistosomiasis is endemic, and Faust, Jones, and Hoffman (56) state that in Puerto Rico the cercariae of *S. mansoni* do produce a prickling sensation while penetrating the skin but a dermatitis does not result.

Vogel (155) found that the cercariae of *S. mansoni* produced a slight dermatitis on the arm of a white subject but not on two negroes exposed in the same manner. Manson-Bahr and Fairley (87) saw cases of dermatitis among Arabs in Egypt which definitely were due to attacks by the cercariae of *S. haematobium* and *S. mansoni*. Lee (71) saw a number of cases among gypsies in the Yangtse River area in China, and Bettencourt *et al.* (6) observed a case on the arm of a woman in Portugal. In each case the cercariae of human schistosomes were known to be present in the water with which the individuals had come in contact or were recovered from snails in the vicinity.

Fülleborn (58) is of the opinion that "kabure" results from the penetration of human skin by non-human schistosome cercariae. Cort (38, 39) is inclined to the view that the cercariae of the so-called human schistosomes seldom produce a significant dermatitis and that cercarial dermatitis is associated with the reaction of an abnormal host to the penetration of a non-specific cercaria. The writer is of the opinion that individual resistance and susceptibility are important factors as some exposures of human beings to non-human schistosome cercariae are negative, while positive cases vary greatly in extent and severity.

It is improbable that all the species of non-human schistosome cercariae can produce a dermatitis in man. The nature of the stimulus to which the organisms give a positive response is not well known and the morphological characters that determine certain aspects of their behaviour are poorly understood. It is not clear how many may be attracted to the human skin but be unable to penetrate it.

At present there are four general types of schistosome cercariae as regards tactic behaviour towards man:

1. Those that do not respond positively to the presence of a human being in the water.
2. Those that respond positively but are unable to penetrate the skin.
3. Those that respond positively and penetrate the skin but, owing to lack of balance between the host and parasite, do not advance deeply and thus provoke only a localized skin reaction.
4. Those that respond positively, penetrate the skin, and eventually make their way to the portal vein and its branches, where sexual maturity is reached. In this case it is thought that frequently no skin reaction occurs.

From the information available it can be assumed that only the cercariae of the eight schistosome species of man belong to group 4. A good many known species may belong to groups 1 or 2, but at the present time only eight species of non-human schistosome cercariae can be placed in group 3. Undoubtedly more will be added in the future, as a schistosome dermatitis is known in several localities where the causal organism has not been definitely identified, or erroneously identified as one of the eight so-called dermatitis provokers.

Those definitely known to provoke dermatitis are as follows:

Cercaria ocellata (La Val. St. George, 1855) Ssintzin, 1909.

C. douthitti Cort, 1916.
C. elvae Miller, 1923.
C. tuckerensis Miller, 1927.
C. stagnicolae Talbot, 1936.
C. physellae Talbot, 1936.
C. pseudoocellata Szidat, 1933.
C. dermolestes sp. nov.

In 1934 the writer incriminated two additional cercariae, both of which were described as new species. *C. bajkovi* McLeod, 1934, is an invalid species, as the characters of two new species were confounded and the description corresponds with neither one. Both species have since been described by Cort and Brackett (42, 43). Similarly the unnoticed production of small numbers of *C. stagnicolae* simultaneously with *C. wardlei* led the writer to assume that the latter was a dermatitis provoker. This work has been rechecked but requires additional confirmation one way or the other.

Furcocercous Cercariae of Manitoba

Seven species of longifurcate pharyngeal cercariae have been recognized in Manitoba, but repeated experiments have proved fairly conclusively that none of them is capable of provoking dermatitis in man. They are as follows:

Species	Manitoba host
Cercaria of *Diplostomum flexicaudum* Van Haitsma, 1931 (*C. flexicauda* Cort and Brooks, 1928)	*Lymnaea stagnalis jugularis* *Stagnicola emarginata canadensis*
Cercaria of *Cotylurus flabellifirmis* Van Haitsma, 1931 (*C. douglasi* Cort, 1917)	*S. emarginata canadensis* *S. palustris elodes*

C. yogena Cort and Brackett, 1937	*S. emarginata canadensis* *S. palustris elodes*
C. dohema Cort and Brackett, 1937	*L. stagnalis jugularis* *S. emarginata canadensis*
Cercaria of *Crassiphiala ambloplitis* Hunter, 1937 (*C. bessiae* Cort and Brooks, 1928)	*Helisoma trivolvis*
C. burti Miller, 1923	*H. trivolvis*
C. wardlei McLeod, 1934	*S. emarginata canadensis*

In addition to the foregoing, three species of brevifurcate apharyngeal cercariae are common in Manitoba, and all three definitely are dermatitis provokers. They are as follows:

Species	Manitoba host
C. elvae Miller, 1923	*L. stagnalis jugularis*
C. stagnicolae Talbot, 1936	*S. emarginata canadensis*
C. dermolestes sp. nov.	*S. palustris elodes*

Descriptions of Cercariae

Cercaria dermolestes sp. nov.

SPECIFIC DIAGNOSIS: An apharyngeal brevifurcate cercaria with a prominent pair of pigmented eyespots. Entire body and tail uniformly spined. Body fusiform with the greatest diameter in the region of the acetabulum. Average measurements of specimens fixed in hot 10% formalin are as follows: Body 226μ in length by 70μ in maximum width; the anterior organ is 68μ in length and the acetabulum is 24μ in diameter and situated 94μ from the posterior end of the body. Tail stem 340μ by 42μ and furcae 210μ in length. Dorso-ventral furcal fin folds are present. Five pairs of penetration glands are present, two being granular and circum-acetabular in position, while the remaining three pairs are post-acetabular in position. These open at the anterior end just lateral to the mouth on the oral sucker by means of fine ducts, the openings of which are located on fine spines. A pair of small escape glands is present in specimens dissected from the sporocyst. Digestive system consists of a tubular oesophagus, which branches in the region of the eyespots into two short blind caeca. The excretory system is composed of seven pairs of flame cells and their ducts. In each lateral half there are three anterior to the acetabulum, three posterior to the acetabulum, and one in the tail stem.

HOST: *Stagnicola palustris elodes* Say.

LOCALITY: Southern Manitoba, Canada.

If the above organism constitutes a legitimate species, this makes a total of four species of cercariae that are identical except for minor metromorphic differences, slight differences in behaviour, and also differences in the choice of snail hosts. They are as follows: *C. elvae* Miller, 1923, *C. stagnicolae* Talbot, 1936, *C. physellae* Talbot, 1936, and *C. dermolestes* sp. nov. The former three were considered as being cospecific for several years but were separated by Talbot in 1936.

All four have the same time of emergence from the snail host, namely 4.30 to 8.00 a.m. According to Talbot (150) and Cort and Talbot (41), *C. elvae* is positively phototactic and in the resting position attaches itself to the wall of the container and assumes a characteristic shape. The body is arched and the tail is bent at an acute angle over the back in the form of a hook with the furcae always crossed.

C. stagnicolae, according to the same authors, is always positively phototactic and hangs suspended from the surface of the water in the region of the greatest light intensity. In this case the body and tail stem are extended and rigid and the furcae are widely separated. They do not, however, attach themselves to the sides of the container.

C. dermolestes sp. nov. is morphologically almost identical with the three species described by Talbot (150) and is of approximately the same size but differs somewhat in relative body measurements. It most closely resembles *C. stagnicolae* Talbot, but the body is shorter, being 226μ on the average, and the tail stem is longer, being 340μ. It resembles *C. stagnicolae* in its behaviour but is a slow sluggish swimmer, and the resting periods are relatively short. At this time it hangs straight down in the water with the furcae only moderately diverged. It has less tendency to bend the body ventrad, so that frequently dorso-ventral mounts can be obtained.

To the writer's knowledge no other schistosome type of cercaria has been recorded from *Stagnicola palustris* except *C. douthitti* and, in view of the fact that at least six species of adult schistosomes occur in Manitoba, this might reasonably belong to a separate species. Larger numbers of this organism can penetrate a thicker barrier of clothing than either of the two other local species and the resulting dermatitis is much more pronounced. Thus, on the basis of morphological and physiological characters, host specificity, and pathogenicity, the writer is of the opinion that the above represents a new species and the name *Cercaria dermolestes* is suggested.

Cercaria wardlei McLeod, 1934 (Fig. 1; Plate I, 3)

This species was recorded from Clear Lake in 1934, but the description, while correct in all major points, is incorrect in one minor point and is not sufficiently detailed to be of any great value in identification. For this reason it is considered advisable to revise and extend the description.

SPECIFIC DIAGNOSIS: A large longifurcate pharyngeal cercaria with a stout body which is pointed at the anterior end, but the sides are almost parallel. Body measurements vary greatly between the largest and the smallest specimens, but the average measurements of a wide range of material fixed in hot 10% formalin are as follows: Body 194μ long by 45μ wide. The anterior organ measures 39μ in length, and the acetabulum which is 41μ in diameter has a distance of 95μ between its anterior margin and the anterior end. The tail stem is 298μ in length by 32μ in width and the furcae are 295μ in length. The oral region is surrounded by a dense band of about six rows of spines, and in the circum-oral spineless area dorsal to the mouth is located a group of

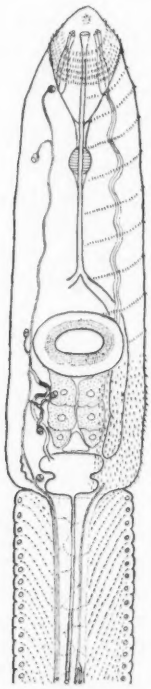


FIG. 1. *Semidiagrammatic sketch of Cercaria wardlei; body and anterior portion of tail stem. About* × *600.*

twelve anteriorly directed spines. The pre-acetabular region bears nine definite rings of spines and the post-acetabular region is uniformly spined. The tail stem bears about ten pairs of long lateral filaments. The pharynx is prominent and the caecal branches reach almost to the posterior end of the body. Three pairs of penetration glands are located posterior to the acetabulum and their ducts open lateral to the mouth. The excretory system is made up of eight pairs of flame cells and their tubules. In each lateral half, two of each anterior group of three are located in the pharyngeal region and the third lies lateral to the acetabulum. The posterior group of three are directed mesad and are fairly uniformly spaced. Two pairs are present in the tail stem and are equally spaced in the second and third fourths. Each lateral collecting tubule has a distinct convolution near its anterior end, and in each arm of this there is a patch of long motile filaments. The excretory bladder is large and has a constriction near its middle, at which point contractions take place during the constant pulsations of the bladder. Caudal bodies are absent, but there are lateral rows of about fifty musculo-epithelial cells in the tail stem. They have distinct nuclei, and the inner muscular portion shows distinct transverse

striations. Such cells are absent or much less prominent in other local cercariae and form an easy and reliable means of identifying *C. wardlei*. A similar type of cell has been reported from cercariae by Ssintzin (139).

C. wardlei is a powerful swimmer, but the swimming periods are short and the organism rotates as it passes along. When agitated it will swim back and forth for a considerable time before becoming quiescent. The resting periods are long and the organism hangs body downward from the water surface with the long furcae widely separated. The pre-acetabular portion of the body may be flexed ventrad or the tail stem may be flexed near its anterior end throwing the body either to right or left.

HOST: *Stagnicola emarginata canadensis*.

LOCALITY: Clear Lake, Manitoba.

Molluscan Hosts in Manitoba

Manitoba lakes and sloughs may be divided into three general ecological groups as regards geographic position, temperature, type of bottom, vegetation, and hydrogen ion concentration. The first, or what may be classed as the oligotrophic type, occurs in the eastern and northern part of the province, involving the Manitoba portion of the Pre-Cambrian shield and commonly known as the eastern region (2). Here the lakes are numerous and are usually very deep with rocky bottoms and very little vegetation. The temperature is low throughout the summer, the oxygen concentration is high, and the pH is neutral or moderately acid. Snails are relatively scarce, and cercarial dermatitis has never been reported from this region, so that it has not been included to any extent in the surveys.

The second or eutrophic type includes the vast majority of Manitoba waters of the prairie region, such as lakes, ponds, and sloughs. Here the water is shallow, somewhat turbid, and the summer temperature is relatively high. The inwash of material is large, the bottom is of loose organic ooze varying in depth and overlying more solid gravel or clay. The pH is on the alkaline side of neutrality. The essential difference between the prairie lake and slough is one of size and also of the amount of vegetation present in the littoral region owing to the differences in water currents.

In the typical prairie slough, plant zonation of the littoral region is fairly distinct. Farthest from the shore may be found such submerged or floating plants as filamentous and other algae, *Elodea* sp., *Potamogeton* sp., *Chara*, *Nitella*, and *Utricularia*. Toward the end of the warm season these may become very dense and reach to the water surface. Emergent vegetation near the shore usually shows more or less definite rings of *Typha*, *Scirpus*, and *Phragmites*. Among the dense vegetation there is usually an abundance of rotifers, nematode worms, crustaceans, snails, and larval insects.

Bays and protected bits of shore line of the larger bodies of water such as Lake Winnipeg, Lake Manitoba, and Lake Dauphin, in general, show much the same fauna and flora as the common slough. In many cases a definite

shore has not yet been formed in these recent lakes, and the body of water shades off into typical marsh or muskeg (Fig. 2; Nos. 11, 12, 14, 20, 21).

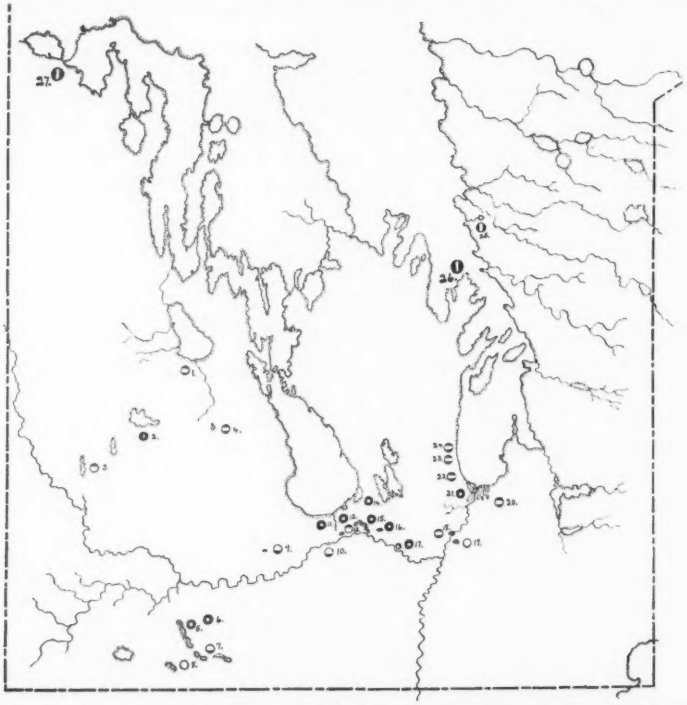


FIG. 2. *Map of southern Manitoba showing districts studied.* ◓ *Cercarial dermatitis known to occur.* ◒ *Molluscs found infested with schistosome cercariae and sporocysts.* ◑ *Districts from which birds have been examined.* ◐ *Districts from which birds were found infested with schistosomes.* 1. *Lake Dauphin*, 2. *Clear Lake*, 3. *Shoal Lake*, 4. *Gilmour Lake*, 5. *Pelican Lake, Ninette*, 6. *Pasture field slough near Ninette*, 7. *Lake Louise, Neelin*, 8. *Killarney Lake*, 9. *Artificial dam near Austin*, 10. *River lagoon, Portage la Prairie*, 11. *Delta marsh*, 12. *Portage Creek*, 13. *Black's Lake, High Bluff*, 14. *Lake Frances*, 15. *Long Lake, Raeburn*, 16. *Pasture field slough near Marquette*, 17. *Pigeon Lake*, 18. *River lagoon, St. Andrews*, 19. *Gravel pits, Birds Hill*, 20. *Libau marsh*, 21. *Netley marsh*, 22. *Whitewold*, 23. *Ponemah*, 24. *Gimli*, 25. *Catfish Creek*, 26. *Blackbear Island*, 27. *Mafeking.*

Clear Lake (Fig. 2; No. 2) represents a type intermediate between the oligotrophic and eutrophic lakes. It is situated at a relatively high altitude in an outcropping of the coniferous forest near the ecotone with the deciduous forest. There is little inwash of organic matter, as sizable inflowing streams are absent. The turbidity of the water is low and the currents are strong. The temperature is low, oxygen concentration high, and pH on the alkaline side of neutrality. The shore is rugged and cold water springs, either with or without definite channels, seep out of the bank in numerous places. The bottom near the shore is of fine sand and coarse stones or boulders. Emergent

vegetation such as reeds or rushes is sparse and confined to localized patches, but a growth of fine algae is found over the surface of the submerged stones.

Birds Hill area (Fig. 2; No. 19) represents an early stage of succession in rain and snow water pools in large gravel pits.

The molluscan fauna of Manitoba is moderately rich in species of Gastropoda, but many of these are few in numbers of individuals and are restricted in distribution (105, 106, 108). No doubt, in future more of these will be found to act as intermediate hosts to schistosomes, but only those that occur where cercarial dermatitis has been reported or suspected are included in the present paper. They are briefly as follows: *Lymnaea stagnalis jugularis* Say, *L. stagnalis perampla* Walker, *Stagnicola palustris elodes* Say, *Helisoma trivolvis* Say, *H. campanulata* Say, *Physella gyrina* Say, *Fossaria obrussa* Say, *Valvata tricarinata* Say, *Amnicola* sp.

L. stagnalis jugularis is one of the largest and most widely distributed snails of Manitoba. It occurs in practically all the prairie lakes and permanent sloughs where the summer temperature is high and the vegetation dense. It was obtained from all the districts studied (Fig. 2) with the exception of 3, 8, 13, 19, and 25, and in every case many were found to be infested with the sporocysts and were liberating cercariae of the dermatitis-provoking type.

S. palustris elodes is a smaller but extremely abundant form found coincidently with *L. stagnalis* in all districts except 9. In addition it was found in 19 and many other small or temporary bodies of water such as ditches, etc. Like *L. stagnalis*, wherever it occurs a sufficient distance from disturbance or human habitation, it is found to be the host to the larvae of schistosome and other types of trematodes.

S. emarginata canadensis, *L. stagnalis perampla*, and *F. obrussa* were found only at Clear Lake. *S. emarginata* is very abundant along the shore of almost the entire lake from a depth of 8 ft. to the water's edge. It is also the common host to the larval stages of a schistosome. Only three specimens of *L. stagnalis perampla* were found and these were uninfested. *F. obrussa* occurs in small numbers in the seepage water from springs around the edge of the lake. Only a few of these were examined, but all were free from trematode infestations. *H. campanulata*, *V. tricarinata*, and *Amnicola* sp. occur in the deeper waters of Clear Lake, but all three species were parasite free.

H. trivolvis and *Physella gyrina* occur in a wide range of situations but both were uninfested with schistosomes. On one occasion specimens of *H. trivolvis* from Pelican Lake were found to be infested with *C. burti* Miller and the cercariae of *Crassiphiala ambloplites* Hunter. An unidentified cercaria, possibly *C. multicellulata* Miller, was obtained on two occasions from *P. gyrina* at Clear Lake.

Primary Hosts in Manitoba

Many species of mammals are found in the vicinity of Manitoba lakes and sloughs and any one or more of these might reasonably act as the primary host of schistosomes. In recent years parasitological surveys of 460 Snowshoe

Rabbits *(Lepus americanus)* and 240 gophers (*Citellus*, 3 sp.) have been carried out in the laboratories of the University of Manitoba (12, 91) but all were negative for schistosomes. Other specimens examined included 36 muskrats (*Ondatra zibethica*) from Clear Lake and Netley and Delta marshes.

Representatives of the majority of Manitoba wild mammals have been examined in the laboratories of the Provincial Game and Fisheries Branch during the past eight years by Dr. J. A. Allan, Pathologist, but as yet no schistosomes have been found.

Five laboratory mice and three kittens were exposed to schistosome cercariae, but post-mortem examination one month later showed them to be free from blood trematodes.

Manitoba is visited annually by large numbers of water fowl either as migrants or summer residents. The ordinary prairie marsh or slough is the feeding and breeding ground of a good many ducks, coots, and grebes, as well as of a number of shore birds and waders. Gulls and terns are fairly common around the lakes and rivers, breeding in either neighbouring marshes or on rocky islands. Loons and merganser ducks nest around some of the deeper lakes but never occur in great numbers. Migrating cormorants, pelicans, and geese, as well as a number of others, remain for varying lengths of time in the spring and fall.

The fact that schistosome cercariae are widespread in Manitoba and that their occurrence coincides with that of the snail host except in certain small areas close to human habitations, indicated that the definitive hosts quite probably would be water birds. This condition, coupled with the fact that over half the known species of schistosomes have water birds as definitive hosts, led to the examination of all possible local species. However, insufficient numbers of some species were examined to give significant results.

Microbilharzia lari was recovered from Herring and Ring-billed Gulls collected at Lake Winnipeg and Clear Lake. Other specimens of Herring and Ring-billed Gulls from the same points as well as other places on Lake Winnipeg, Lake Winnipegosis, and Clear Lake yielded two additional species of schistosomes, namely: *Ornithobilharzia aviani* sp. nov. and *Ornithobilharzia filamenta* sp. nov.

Both species of gulls occur in considerable numbers around the larger Manitoba lakes and their tributaries. They also occur in moderate numbers at Clear Lake. A few loons and merganser ducks are found at Clear Lake, but ordinary pond or diving ducks are scarce during the breeding season or late summer. Blue-winged Teal and Golden-eye Ducks frequently alight and feed for a short time near the water's edge in the early morning.

The Blue-winged Teal is the commonest duck nesting around prairie lakes and sloughs. They are very numerous and widely distributed; over 60% of all specimens, both adult and juvenile, were infested with the males of *Pseudobilharziella querquedulae* McLeod, 1937. These were collected from a good many localities, and infested birds were encountered in every case regardless of the season.

TABLE I

BIRDS EXAMINED FOR SCHISTOSOMES

Specific name	Common name	No. examined	No. infested
Larus pipixcan	Franklin's Gull	7	0
Larus argentatus	Herring Gull	32	7
Larus delawarensis	Ring-billed Gull	20	5
Sterna hirudo	Common Tern	6	0
Dafila acuta	Pintail Duck	12	0
Querquedula discors	Blue-winged Teal	38	22
Nettion carolinense	Green-winged Teal	4	0
Anas platyrhynchos	Mallard Duck	9	0
Nyroca valisneria	Canvas-back Duck	3	1
Spatula clypeata	Spoonbill Duck	12	0
Mareca americana	Baldpate Duck	1	0
Nyroca marila	Bluebill Duck	3	0
Nyroca americana	Redhead Duck	30	0
Nyroca collaris	Ringneck Duck	1	0
Erismatura jamaiciensis	Ruddy Duck	2	0
Glaucionetta clangula	Golden-eye Duck	1	0
Branta canadensis	Canada Goose	1	0
Fulica americana	Coot	7	0
Colymbus nigricollis	Eared Grebe	3	0
Limosa fedoa	Marbled Godwit	1	0
Phalacrocorax auritus	Crested Cormorant	1	0
Bartramia longicauda	Upland Plover	1	0
Gavia stellata	Red-throated Loon	4	0
Gavia immer	Northern Loon	1	0
Ceryle alcyon	Belted Kingfisher	1	0
Botaurus lentignosus	American Bittern	1	0
Mergus americanus	American Merganser	4	0
Pelecanus erythrorhynchus	White Pelican	1	0

Total number of species 28. Total number of specimens 207.

The Canvas-back Duck is common in southern Manitoba only during the seasonal migrations. A single specimen infested with *Microbilharzia manitobensis* McLeod, 1936, and *Microbilharzia canadensis* McLeod, 1936, was collected in late fall at Lake Frances.

An attempt was made to infect several species of wild ducks experimentally with each of the three species of schistosome cercariae. Hatchery-reared birds of from one to six weeks of age were obtained and some of each species were exposed repeatedly in the laboratory for intervals of from 15 to 45 min. to water containing the cercariae. In other cases thick infusions of cercariae were injected intravenously, intraperitoneally, or subcutaneously by means of a hypodermic needle.

Birds Exposed Experimentally

Specific name	Common name	Number
Anas platyrhynchos	Mallard Duck	5
Dafila acuta	Pintail Duck	5
Nyroca valisneria	Canvas-back Duck	5
Nyroca americana	Redhead Duck	4
Querquedula discors	Blue-winged Teal	10
Anas boschas domestica	Domestic Duck	6

Control birds were kept in each case in order to check the accuracy of the experiment. One domestic duck exposed to *C. stagnicolae* developed a severe dermatitis on the second day and died on the sixth day as a result. Post-mortem examinations were carried out up to six weeks after exposure but all were negative.

Classification of the Schistosomatidae

The discovery of several new species of schistosomes in the past few years, particularly those recorded by Ejsmont (47), Wetzel (158), and the writer (93, 94), necessitates a revision of the classification and also the keys for the identification of the various members of the family. In the following keys Price's classification (114) is used as a basis, but a number of minor changes are suggested.

Price (115, 116) suggests the re-establishment of the genus *Macrobilharzia* Travassos, 1923, to accommodate *Paraschistosomatium anhingae* Price, 1929, and *Ornithobilharzia macrobilharzia* (Travassos, 1923) Price, 1929, and suggests that the genus *Paraschistosomatium* Price, 1929, be deleted. This suggestion has been adopted in the following classification.

The genus *Ornithobilharzia* Odhner, 1912, should be amended, as suggested by Wetzel (158), to include forms in which the female is shorter or longer than the male, having 28 to 120 testes in the male, and also having the spiral ovary of the female confined to the anterior one-third of the body. This would necessitate the amending of the genus *Microbilharzia* as follows: Suckers present in the male, present or absent in the female. Testes 18 to 26 in number in the male, ovary pre-equatorial in the female and in the form of a loose spiral. The species *Ornithobilharzia lari* McLeod, 1937, would then be transferred to the genus *Microbilharzia*. The creation of the new genus *Pseudobilharziella* by Ejsmont (48) calls for a revision of the generic diagnosis of the old genus *Bilharziella*, and he suggests the following changes: Anterior region narrow and one-half of the body length; posterior region broad and lanceolate. Genus *Pseudobilharziella:* Anterior portion of the body broad and one-fifth of the body length; posterior portion narrow and strap-like.

A modification of Price's classification in accordance with the above suggestions consequently would be as follows:

Key to the Sub-families of the SCHISTOSOMATIDAE

Females slender, more or less cylindrical in cross-section; males larger than females, flattened, and with the lateral edges infolded forming a gynaecophoric canal; intestinal caeca usually unite caudad of the equator of the body; testes situated cephalad of the caecal union . . . SCHISTOSOMINAE.

Females similar to males in shape; males without well developed gynaecophoric canal; caecal branches unite cephalad of the equator of the body; testes situated caudad of the caecal union . . . . . BILHARZIELLINAE.

KEY TO THE GENERA OF SCHISTOSOMINAE Stiles and Hassal, 1898

1. Females unknown; male gynaecophoric canal well developed; testes 70 to 83 in number, in posterior third of body and cephalad of the caecal union; in mammals ..... *Heterobilharzia.*

   Males and females both known ..... 2.

2. Testes 230 to 250 in number, from just caudad of acetabulum into posterior half of body; female slender, much shorter than male; ovary spiral, in the posterior third of the body; intestinal caeca unite in posterior end of body; uterus long and filled with eggs ..... *Macrobilharzia.*

   Gynaecophoric canal well developed; testes less than 120 ..... 3.

3. Testes 28 to 120 in number, in the anterior two-thirds of the body; female shorter or longer than male; ovary spiral in anterior one-third of body ..... *Ornithobilharzia.*

   Testes 26 or less in number; ovary just pre- or post-equatorial in position; suckers present in male, present or absent in female ..... 4.

4. Anterior end of gynaecophoric canal near equator of body; testes in two rows, at anterior end of gynaecophoric canal; genital pore immediately in front of anterior testis; intestinal caeca with short lateral diverticula; common caecum in both sexes short; ovary pre-equatorial ..... *Schistosomatium.*

   Anterior end of gynaecophoric canal near acetabulum; genital pore of male a short distance caudad of acetabulum; intestinal caeca with diverticula reduced or absent; common caecum usually long; ovary pre- or post-equatorial in position ..... 5.

5. Testes less than ten in number; ovary oval ..... *Schistosoma.*

   Testes 18 to 26 in number; ovary spiral ..... 6.

6. Anterior end of gynaecophoric canal slightly caudad of acetabulum; oral sucker lacking in female; ovary about one-third of body length from the posterior extremity ..... *Austrobilharzia.*

   Anterior end of gynaecophoric canal cephalad of acetabulum; suckers present or absent in female; ovary pre-equatorial ..... *Microbilharzia.*

KEY TO THE GENERA OF BILHARZIELLINAE Price, 1929

1. Body cylindrical or nearly so ..... 2.

   Body flattened ..... 3.

2. Female unknown; posterior end of body threadlike, middle portion wider than either the anterior or posterior portions; no gynaecophoric canal; suckers present ..... *Trichobilharzia.*

   Male and female very long and slender; gynaecophoric canal reduced to a short groove in the anterior part of the body; suckers absent ..... *Gigantobilharzia.*

3. Suckers absent; common caecum with short lateral dendritic branches ..... *Dendritobilharzia.*

   Suckers present; common caecum without lateral dendritic branches ..... 4.

4. Body divided into narrow anterior and broad lanceolate posterior portions, the point of union being at the junction of the second and third fifths of the body..... *Bilharziella.*

Body divided into broad anterior and narrow strap-like posterior portions, the point of union being at the junction of the first and second fourths of the body..... *Pseudobilharziella.*

KEY TO THE SPECIES OF THE GENUS MICROBILHARZIA Price, 1929

1. Testes in male reaching into post-equatorial region.....2.

Testes in male confined to pre-equatorial region.....3.

2. Body fairly stout in male; caecal branches slightly convoluted and without diverticula; female unknown..... *M. chapini.*

Body slender in male; caecal branches convoluted and with small diverticula; female unknown..... *M. manitobensis.*

3. Caecal branches in male uniting at about the junction of the middle and posterior thirds of body; common caecum long; acetabulum wanting in female..... *M. canadensis.*

Caecal branches in male uniting near hinder end of body; common caecum short; acetabulum present in female..... *M. lari.*

ADULT SCHISTOSOMES OCCURRING IN MANITOBA

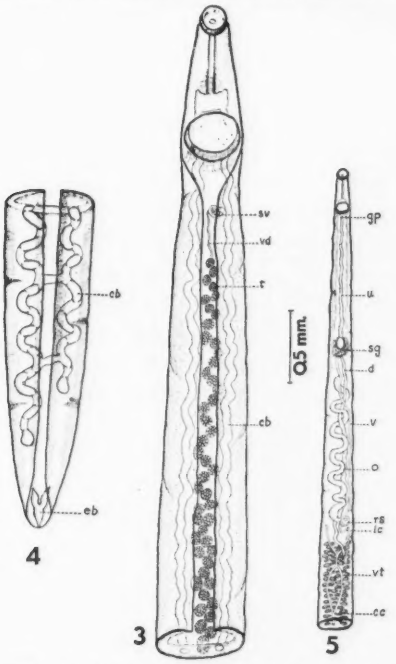
Species	Host
Microbilharzia canadensis McLeod, 1936.	*Nyroca valisneria.*
M. manitobensis McLeod, 1936.	*N. valisneria.*
M. lari McLeod, 1937.	*Larus argentatus.*
Pseudobilharziella querquedulae McL. 1937.	*Querquedula discors.*
Ornithobilharzia aviani sp. nov.	*L. argentatus.*
O. filamenta sp. nov.	*L. delawarensis.*
	L. argentatus.

Descriptions of New Species

Ornithobilharzia aviani sp. nov. (Figs. 3–5)

GENERIC DIAGNOSIS: *Ornithobilharzia.*

MALE: Large straight-bodied worms of an average length in fixed specimens of 12 to 15 mm. and of a fairly uniform diameter, reaching a maximum of 0.83 mm. The lateral edges of the body are infolded to form a well developed gynaecophoric canal which begins just caudad of the acetabulum and gradually becomes more pronounced. It is only moderately deep throughout most of its length and gradually disappears near the caudal end. The edges normally do not meet in the mid-line. The cuticle is thick and without tubercles or spines. The oral sucker is terminal and in the form of a shallow funnel, the aperture of which is directed oblique ventrad. It is circular and has an average diameter of about 250 μ. The anterior body portion is elliptical in



FIGS. 3–5. *Ornithobilharzia aviani sp. nov.* FIG. 3. *Anterior portion of male.* FIG. 4. *Posterior end of male.* FIG. 5. *Anterior portion of female.* cb. *caecal branch;* cc. *common caecum;* d. *vitelline duct;* eb. *excretory bladder;* gp. *genital pore;* lc. *Laurer's canal;* o. *ovary;* rs. *receptaculum seminis;* sg. *shell gland;* sv. *seminal vesicle;* t. *testes;* u. *uterus;* v. *vagina;* vd. *vas deferens;* vt. *vitellaria.*

cross-section and the acetabulum is pedunculate. Its anterior margin is 0.82 mm. from the anterior end of the body and the average diameter is 400μ. The mouth is situated in the centre of the oral sucker and leads into a narrow straight tube. A short distance back, it passes through a mass of tissue, partly muscular, partly glandular in nature. Cephalad of the acetabulum, it passes into a squarish cavity of considerable size. The caecal branches arise from the posterior-lateral margins of this and follow a slightly convoluted course caudad. Farther back, the caeca become progressively larger and more convoluted and later, anastomosis occurs. There are some individual variations near the posterior end where there may be one or two anastomosing branches. Following this, the caeca may be of unequal length and end singly or may unite, the common caecum ending close to the posterior end of the body. A small but distinct Y-shaped excretory bladder opens at the posterior tip of the body. The testes are arranged in a row, which at the anterior end is convoluted, while toward the hinder part it is made up of a compact mass about two testes in width. The row begins about 0.68 mm. caudad of the acetabulum and extends into the post-equatorial region. In mature speci-

mens, the testes are spherical, about 95μ in diameter, and vary from 54 to 74 in number. The vas deferens is narrow and moderately straight. It opens into a transversely elongated seminal vesicle. The genital pore is considerably to the left of the median line and is about 0.28 mm. caudad of the acetabulum. A prostate or cirrus pouch is absent.

FEMALE: Filamentous worms considerably shorter than the males and usually completely enclosed in the gynaecophoric canal. The average length in fixed specimens is 7.2 mm. by 0.23 mm. in width. The portion anterior to the ovary is cylindrical and finely attenuated, while the hinder portion is flattened and bluntly pointed. The cuticle is finely tuberculate. The oral sucker is terminal and has an average diameter of 50 μ. The acetabulum is well developed and pedunculate, with a diameter of 63 μ. The digestive canal branches into two fine caeca just cephalad of the acetabulum. These pass caudad to just behind the ovary where they may unite permanently or separate and reunite once or twice. The common caecum follows a straight course to near the hinder end of the body. The ovary is a spiral consisting of about eight loops and begins 1 mm. from the acetabulum. Its length, disregarding the total length of the spiral, is 0.78 mm. A small receptaculum seminis is present at the posterior end of the ovary and Laurer's canal is also present. An oviduct of moderate size passes forward to the ootype, which is distinct and located 0.81 mm. caudad of the acetabulum. The vitellaria are in the form of numerous small oval follicles, which occupy most of the region caudad of the ovary. The vitelline duct passes forward in company with the oviduct to the ootype. The uterus is narrow, moderately straight and the genital pore opens behind the acetabulum. Eggs are produced singly.

CERCARIA: Unknown or unrecognized.

TYPE HOST: Primary, birds *(Larus argentatus)*, secondary, unknown.

TYPE LOCALITY: Lake Winnipeg, Manitoba, Canada.

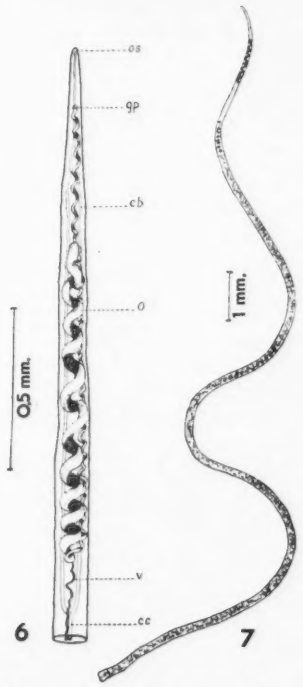
LOCATION: Portal and intestinal veins.

The above description is based on the examination of a few pairs of males and females in a good state of preservation, taken in copula from the intestinal veins of an adult specimen of *Larus argentatus*. Both males and females closely resemble those of *O. canaliculata* (Rudolphi, 1819) Odhner, 1912. The male also resembles that of *O. kowalewskii* (Parona and Ariola, 1896) Odhner, 1912, in so far as the brief descriptions of these two species go. Dr. Price suggests that there is a strong possibility of the two being cospecific, but because of the brevity of the published descriptions, it is impossible to establish the point. The writer considers it advisable to regard the above as a new species until such time as specimens of both *O. canaliculata* and *O. kowalewskii* can be examined in detail and the differences of similarities determined for all three.

Ornithobilharzia filamenta sp. nov. (Figs. 6 and 7)

GENERIC DIAGNOSIS: *Ornithobilharzia.*

MALE: Large straight-bodied worms of an average length in fixed specimens of 12 to 15 mm. and of a fairly uniform diameter reaching a maximum of 0.63 mm. The lateral edges of the body from just behind the acetabulum to very near the posterior end are infolded to form a fairly well developed gynaecophoric canal. It is only moderately deep in the mid-body region and becomes



FIGS. 6 and 7. *Ornithobilharzia filamenta sp. nov.* FIG. 6. *Anterior portion of female.* FIG. 7. *Entire female.* cb. *caecal branch;* cc. *common caecum;* gp. *genital pore;* o. *ovary;* os. *oral sucker;* v. *vitelline duct.*

progressively shallower toward the posterior end. The edges normally do not meet in the mid-line. The cuticle is thick and without tubercles or spines. The oral sucker is terminal and in the form of a shallow funnel, the aperture of which is directed obliquely ventrad. It has a length of about 210 μ and a width of about 205 μ. The anterior portion of the body is elliptical in cross-section and the acetabulum is pedunculate. Its anterior margin is about 0.81 mm. from the anterior end and the average diameter is 330μ. The mouth is situated in the centre of the oral sucker and leads into a narrow straight tube. A short distance back it passes through a mass of dense,

deeply staining tissue and anterior to the acetabulum it passes into a bulbous cavity of considerable size. The caecal branches arise from the posterior-lateral margins of this and follow a convoluted course caudad. Toward the posterior end, they become progressively larger with traces of secondary branches and show a tendency to anastomose. In certain cases they unite to form a common caecum, but in others they unite by cross branches and each ends blindly. A small distinct Y-shaped excretory bladder opens at the posterior tip of the body. The testes are arranged in an irregular single row, which follows a zig-zag course. The row begins about 0.7 mm. behind the acetabulum and extends to within about 4.4 mm. of the posterior end. In mature specimens the testes are oval, 89 μ in diameter, and vary in number from 88 to 112 in different individuals. The vas deferens is narrow and moderately straight. It opens into a transversely elongated seminal vesicle. The genital pore is considerably to the left of the median line and is about 0.28 mm. behind the acetabulum. A prostate or cirrus pouch is absent.

FEMALE: Extremely long and filamentous, with a length of 15 to 20 mm. and a maximum width of 0.1 mm. A short anterior portion of the body is cylindrical and finely attenuated, while the remainder is broader with parallel sides, flattened, and ends bluntly. The oral sucker is feebly developed and is 16 μ wide. It is conical in shape and the aperture is subterminal in position. An acetabulum is entirely lacking and the cuticle is finely spined. The ovary is an elongated tubular body forming a spiral of about 8 to 9 turns and lies in the middle of the anterior one-eighth of the body, beginning about 0.61 mm. from the anterior end. The vitellaria are in the form of a single row of numerous relatively large follicles, each of which is almost as wide as the body. They occupy the posterior seven-eights of the body. The oviduct passes forward from the posterior end of the ovary, and the uterus appears to open a short distance from the anterior end. The ootype and Mehlis' gland were not discernible and eggs were not present in the female worms. The alimentary canal is simple, branching midway between the oral sucker and the ovary, and the caecal branches reunite just posterior to the ovary. The common caecum is long, reaching almost to the posterior end and following a zig-zag course.

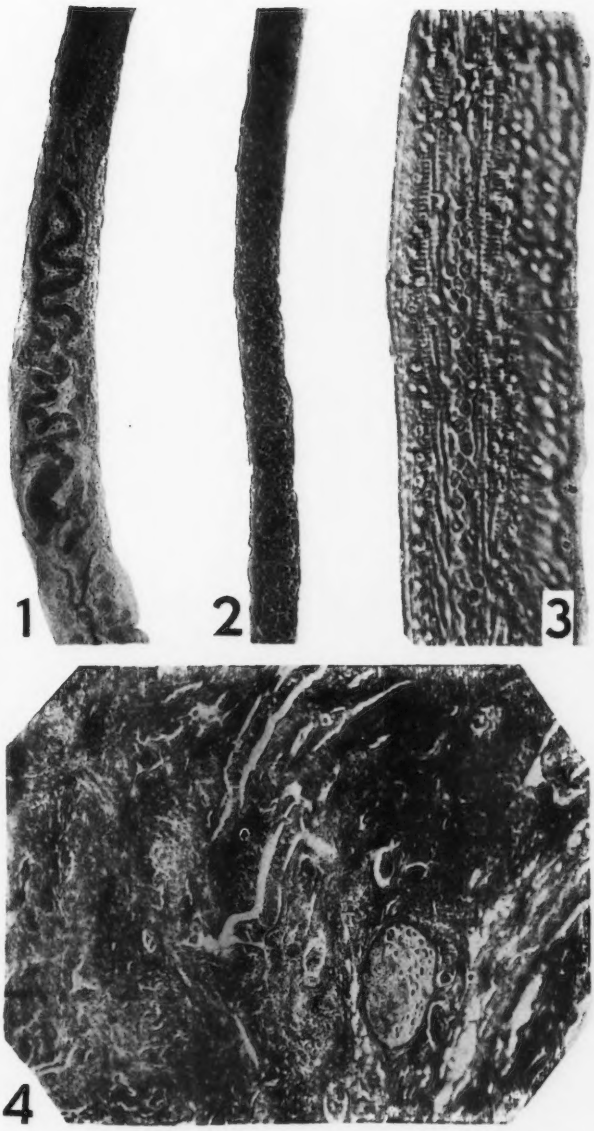
CERCARIA: Unknown or unrecognized.

TYPE HOST: Primary, Birds *(Larus delawarensis* and *L. argentatus)*; secondary unknown.

LOCALITY: Lake Winnipeg and Clear Lake, Manitoba, Canada.

LOCATION: Coccygeomesenteric and mesenteric veins and lymphatics.

The male of *Ornithobilharzia filamenta* is almost identical morphologically with that of *O. aviani*, except for the number of testes present. The two species occur coincidently in the same host, so for this reason, only males actually in copula with the females have been used in determining the specific characters. The female differs greatly in size and also in the shape and position of the genitalia from that of *O. aviani*, and thus serves to distinguish the two



1. *Photomicrograph of genital region of female Ornithobilharzia aviani sp. nov., showing spiral ovary, seminal receptacle, caecal union, oviduct, shell gland and vitellaria,* × *60.*

2. *Photomicrograph of mid-body region of female Ornithobilharzia filamenta sp. nov. showing single row of large vitellaria,* × *60.*

3. *Photomicrograph showing tail stem musculature of Cercaria wardlei,* × *940.*

4. *Photomicrograph of section of lymphatic from infested Herring Gull showing sections of female Ornithobilharzia filamenta sp. nov. and fibrosed cyst containing what appears to be eggs,* × *20.*

species at a glance. *O. filamenta* has been found in the hepatic portal and mesenteric veins but appears to prefer the coccygeomesenteric vein, as females frequently are found there in large numbers. In some cases they are found to have migrated into the neighbouring lymphatics and produced granular, tumour-like swellings of considerable size. Histological sections of one of these tumours (Plate I, 4) show sections of several female worms and also what is taken to be groups of eggs that have been walled off by fibrous tissue. Lesions of the gut wall have not been found and free eggs have not been seen.

The males of the above described species, as in *O. aviani*, are very similar to those of *O. canaliculata* and *O. kowalewskii*. The female, in general, resembles that of *O. intermedia* and that of *O. pricei* but is much longer, the ovary is relatively farther forward, and the acetabulum is lacking entirely. The characters of the gynaecophoric canal and testes in the male and the ovary and vitellaria in the female definitely place the above organism in the genus *Ornithobilharzia*. It differs, as pointed out, particularly in the case of the female, from any described species and is submitted as being new to science. The name *Ornithobilharzia filamenta* is suggested.

Discussion

A total of 15 species of adult schistosomes has been recorded from the United States and Canada. One of these *(B. polonica)* had been described previously from other countries and the life-history worked out in detail, but the cercariae have not been reported from North America. Two of the 14 species considered as being native to North America have their adult stages in mammals (rodents), and in both species the life-history is known and the cercariae have been described and their distribution studied. The remaining 12 have been reported as adults from birds and the cercarial stages are either unknown or unrecognized. Whether the infestations are acquired in these two countries or in some other is, as yet, undetermined. Six distinct species of schistosome cercariae are thought to occur in North America, and no doubt several more will be discovered in the near future or are being mistaken at the present time for one or more of the recorded species.

Six species of adult schistosomes have been found in Canada by the writer, and all these occur in migratory birds in the southern half of Manitoba. Two species are rare, having been found only once in the Canvas-back Duck, a bird that occurs here only as a migrant.

Pseudobilharziella querquedulae is the commonest schistosome found locally. It occurs in 60% of all the specimens of *Querquedula discors* examined, regardless of the season or the place from which the birds were collected. *P. querquedulae*, undoubtedly, is the adult of one of the three local dermatitis-provoking cercariae, as immature worms have been recovered from fledgling birds that were not yet able to fly but had frequented waters of prairie lakes, where both *Cercaria elvae* and *C. dermolestes* were abundant.

It is remarkable that no females of this species have been found in so large a number of examinations in which males were plentiful. There is the

possibility that they have been overlooked on account of their small size. A more logical explanation, perhaps, is that *Q. discors* is an unnatural host and only the male schistosomes develop in it. This was found to be the case by Faust (53) in infections of human schistosomes in unnatural hosts such as the rabbit and sheep.

Microbilharzia lari and *Ornithobilharzia aviani* are found in local adult Herring Gulls in fairly large numbers. It is not known where the infection is contracted, there being no more evidence to favour the winter feeding ground than the summer habitat. *Diplostomum flexicaudum* is carried in the adult stage by the Herring Gull, and the larvae occur coincidently with schistosome cercariae at Clear Lake. This might indicate that *C. stagnicolae* is the larva of one of the gull schistosomes. Immature male worms of both species were obtained from birds collected in late autumn and, as the time required for maturation in most schistosomes is usually three months or less, this would strengthen the theory that the infection occurred in local waters.

O. filamenta was found in both Herring and Ring-billed Gulls from Clear Lake in midsummer and from Lake Winnipeg in midsummer as well as late autumn. Mature and immature males and females were recovered and the above argument would apply equally to these.

Consistent failure to obtain adult worms by exposure of experimental animals may be due to a number of causes. In addition to the experimental work by the writer, Talbot (150) exposed young domestic ducklings, Herring Gulls, and a pigeon to three species of schistosome cercariae from Douglas Lake, Michigan, but the results were negative in every case. Differences in the temperature, the pH, or the salt concentration of the water used in the laboratory may have been responsible for the production of cercariae so inviable that they were unable to penetrate the skin of experimental animals, or these changes may have interfered with their normal responses to the presence of a host, although this seems unlikely.

Effective treatment of cercarial dermatitis once it has been contracted by human beings is very difficult. The intense itching and annoyance are the most serious aspects, and treatment is usually directed to relieve these. Application of various lotions or ointments is usually recommended, but the writer has found them to be of little or no value in either reducing the irritation or killing the organisms.

The direct pathological effects of schistosomes on the bird host is not known in any detail, but infested birds are invariably lighter and more emaciated than uninfested ones. Much additional work is required on the life-histories and pathogenicity of the local forms.

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THE HELMINTH PARASITES AND PARASITIC DISEASES OF SHEEP IN CANADA

I. A SURVEY AND SOME PRELIMINARY STUDIES ON EXISTING PROBLEMS[1]

By W. E. Swales[2]

Abstract

Investigations have been made upon the identity, incidence, economic importance, and seasonal fluctuations of the helminth parasites in the alimentary canal of sheep in Canada, special consideration having been given to the problems in Quebec and eastern Ontario. Haemonchiasis is the parasitic disease of major importance in Eastern Canada during the summer months, and is replaced by trichostrongylosis, oesophagostomiasis, and possibly hookworm disease in the late autumn and winter. Haemonchiasis and chabertiasis were recorded in British Columbia.

Introduction

Sheep husbandry has not flourished in Canada. At the present time there are no more sheep in the Dominion than there were in 1871, and at that time the important sheep ranges in the Prairie Provinces were not being used. The number of sheep in Ontario in 1937 was 874,769, compared with 1,915,303 in 1882. The decrease in Quebec over the same period was not as great, and the number has approximated 750,000 for the last ten years.

The lack of expansion and actual decrease in sheep production in Eastern Canada have been due to a number of causes, chief among which are probably the difficulty of accommodating and feeding breeding stock during the winter months, a period of low prices obtained for meat and wool, high costs of fencing materials, lack of protection against marauding dogs and predatory animals, increased need of available pastures near cities for dairy cattle, and disease. However, in many outlying areas in Eastern Canada large tracts of land are unsuitable for the production of dairy products but could be adapted for sheep production on a small unit basis, and would produce this "cash crop" with a minimum of expenditure or labour. In those areas the problems of fencing and winter accommodation could be overcome by the present availability of cheap labour and lumber. Shipping costs are now comparatively low, predators are fewer in numbers and prices for good market animals have steadily improved. Canada has been fortunate in having a very low incidence, and in many cases complete absence, of such important bacterial, virus, and protozoal diseases of sheep as anthrax, foot and mouth disease, louping ill, necrobacillosis, variola ovina, heart-water, piroplasmosis, scrapie, and braxy. Psoroptic mange has not been allowed to become established and calliphorine

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myiases are comparatively uncommon. Haemorrhagic septicaemia must be considered as an important problem, although some preventive measures can be applied during periods of exposure. Contagious ecthyma appears to be of moderate importance in range flocks, but is unlikely to become prevalent under eastern conditions.

Diseases due to internal parasites appear to be major causes of losses, particularly in Eastern Canada. Owing to climatic extremes, species that are normally distributed in temperate climates and some that are usually considered as being important only under semi-tropical conditions, are enabled to complete their life cycles on pastures in the Eastern Provinces. It is thought by competent authorities that parasitic disease has been and is still the greatest deterrent to profitable production of sheep in many parts of the Dominion. Extension work has reduced losses considerably in some agricultural districts, particularly in Ontario; the methods of control generally employed have been those in use in the United States of America, and have been directed chiefly against verminous gastritis in lambs due to infestations of *Haemonchus contortus*. However, discouraging losses and lack of thriftiness in lambs and breeding stock have continued throughout many areas, particularly in Eastern Canada.

In the hope that a thorough investigation of the incidence, economic importance, and control of the parasites of sheep in Canada would contribute towards more successful sheep husbandry and would encourage farmers to extend this industry, a series of studies was commenced in 1936. The present paper represents the first of the series, and includes briefly the results of the preliminary work.

The Occurrence of Species

The following species have been found in sheep and lambs in Canada, in natural infections, during the course of these studies.

Parasite	*Estimated Prevalence*
CLASS—TREMATODA	
Dicrocoelium dendriticum	Recorded in Nova Scotia only.
Fasciola hepatica	Confined to small areas.
CLASS—CESTODA	
Moniezia expansa	Very common.
Thysanosoma actinioides	Common in Western Canada.
Cysticercus tenuicollis	Very common.
C. ovis	Rare.
CLASS—NEMATODA	
Ascaris sp.	Rare.
Strongyloides papillosus	Very common.
Oesophagostomum columbianum	Very common in Eastern Canada.
O. venulosum	Common in British Columbia.
Chabertia ovina	Very common.
Monodontus trigonocephalus	Very common.
Dictyocaulus filaria	Not common.
Muellerius capillaris	Very common.
Trichostrongylus axei	Very common.

Parasite	*Estimated Prevalence*
CLASS—NEMATODA—*conc.*	
T. vitrinus	Very common.
T. colubriformis	Very common.
Cooperia curticei	Very common.
C. oncophora	Not common.
Ostertagia circumcincta	Very common.
Haemonchus contortus	Very common.
Nematodirus filicollis	Very common.
N. spathiger	Common.
Gongylonema pulchrum	Rare.
Trichuris ovis	Very common.
Capillaria longipes	Not common.

The above list includes only two additions to other published records in Canada (Swales (12) and Fallis (5)), these being *Gongylonema pulchrum* and *Capillaria longipes*. It is, therefore, considered to be an accurate list of all species that are liable to be encountered in reasonably large samples. The expected discovery of other species of *Trichostrongylus* and *Ostertagia* has not been made.

Special Technique Employed in Autopsies

In estimating the numbers of individuals of the larger species, *Haemonchus contortus*, *Moniezia* spp., *Monodontus trigonocephalus*, *Trichuris ovis*, *Chabertia ovina*, and *Oesophagostomum columbianum*, direct counts of all individuals screened from intestinal washings were made. In light infections *Ostertagia circumcincta* and *Nematodirus* spp. were also counted individually. In order to estimate the numbers of the small species, *Trichostrongylus* spp., *Cooperia* spp., and *Strongyloides papillosus*, it was necessary to employ a dilution technique.

Owing to the importance of making accurate estimations the examination of a small portion of a formalin suspension of the intestinal contents was not found adequate. After numerous tests a simple technique was evolved which has proved to be a reliable and rapid method, and which has been adopted as the standard technique in these studies. This method is described in some detail owing to the interest expressed in it by several visiting parasitologists.

After removal from the animal the abomasum is ligated at the omaso-abomasal orifice and at the pylorus, the duodenum being left attached to the abomasum. The ileum is ligated at or near the ileo-caecal valve. The whole is then immersed in 5 to 10% formalin in a tank large enough to conform to the rules necessary for adequate fixation; specimens are left in this solution for at least two days.

The preserved abomasum is examined by opening it over a pail, and a stream of water is run on to the mucous membrane as it is thoroughly rubbed with the fingers. The suspension of ingesta and parasites is then screened through a stack of sieves (Fisher's U.S. Standard Sieve Series Nos. 10, 20, and 40) which have mesh diameters or nominal openings of 2.00 mm., 0.84 mm., and 0.42 mm. respectively, and with wire diameters of 0.76 mm., 0.42 mm., and

0.25 mm., and which are 21.7 cm. in over-all diameter. The type of sieve as devised by Hall and Cram is not suitable for this method.

As the sieves are filled, a well-fitting cover is placed over them and they are shaken with a rotary motion. Water is added until most of the soluble material is removed. Each sieve is now inverted in a large moist chamber dish made of thick glass and the contents are washed off with water which has been cleared of air bubbles. The contents of each dish are allowed to settle and the supernatant water is then decanted. The large species are now counted, the dish being placed over a black surface and under a strong light, for this purpose. The contents of the dish are then transferred to another dish which has a diameter of 25 cm. and a bottom area of 491.0 sq. cm. The contents, which should be approximately half an inch in depth, are gently shaken until they are evenly distributed in the dish; a metal ring is quickly placed in the mass in such a way that its edge is near the centre of the dish. This ring, which has an area of 49.1 sq. cm. thus separates one-tenth of the contents of the dish, and this amount is pipetted into a square Petri dish which has been ruled to fit the field of a dissecting microscope. The ring used in this work is the screw-top of a one-pint glass preserving jar, the bottom edge having been levelled and sharpened.

The one-tenth sample from each dish is then examined systematically under a magnification of $\times 25$ for the detection and identification of the genera of small parasites.

In 20 tests with 1,000 adult *Ostertagia circumcincta*, this sampling technique indicated the number within 10% in 19 tests, the mean error being $\pm 4.45\%$.

This technique requires some practice and experience in spreading the ingesta in the dish and placing the ring, in order to obtain accurate results, but its use approximately doubled the number of specimens that it was previously possible to record by the use of other reasonably accurate methods.

Observations on the Abundance and Pathogenicity of Parasites in Lambs in Eastern Canada

In 1936 a preliminary survey of the parasites in 134 abomasa of market lambs was made. The lambs came from farms in the southern sections of Quebec and in eastern Ontario. The collections were made at random in abattoirs in Montreal. The results of the examinations of the contents, made under laboratory conditions, are shown in Table I.

The low numbers of individuals recovered from these animals would be due, in part at least, to the fact that the lambs were relatively healthy specimens and were slaughtered between October 23 and November 25.

As noted by Fallis (5) and as illustrated later in this paper, the peak of abundance of *H. contortus* occurs in the summer and then the number of individuals decreases rapidly in the months of autumn. *O. circumcincta* has a similar fluctuation, but *T. axei* occurs in greatest numbers during the late autumn or winter months.

TABLE I

ABOMASAL PARASITES OF 134 LAMBS FROM QUEBEC AND EASTERN ONTARIO

Parasite	No. of lambs infected	Percentage infected	Mean no. of worms per infected lamb	Min. and max. nos. of worms
Haemonchus contortus	82	61.2	45.4	1 – 530
Ostertagia circumcincta	99	73.9	154.2	3 – 1,620
Trichostrongylus axei	124	92.5	605.4	20 – 8,710

The results of these preliminary observations on abomasal parasites agree in all important details with those of Fallis in Ontario, seasonal variations being considered, and for this reason no further details are included.

The degree of infection with certain species of nematodes which is able to cause clinical disease or at least mark unthriftiness in lambs or adult sheep, has been the subject of some study. An examination of the literature reveals that some workers have set a tentative line between tolerated and harmful infections for known pathogenic species. It is usually considered that mixed infections are more harmful than infections with a single species, and this supposition, supported by some evidence of lowered resistance to one species being produced by another, will remain for the present a complication in preliminary observations on pathogenicity. Ross and Gordon (10) state that it is highly improbable that infections with less than 500 *H. contortus* are ever the primary cause of serious effects, and that fatal cases are seldom seen with less than 1,000 individuals of this species. They point out the importance of the complicating factor of nutrition in such estimation.

Robertson (9) estimates that fatal results are not produced by less than 8,000 individuals of *O. circumcincta*. Taylor's (14) estimations of numbers of this species would indicate that fatal results are produced by this species, but that about 16,000 individuals are necessary.

Trichostrongylus spp. are the undoubted cause of severe gastro-enteritis in Great Britain, Africa, and Australia, and under suitable climatic conditions must always be considered as potential pathogens. Ross and Gordon state that fatal infections are seldom induced in Australia by less than 10,000 of these worms in the abomasum and small intestine, and state at the same time that Taylor's work indicates that in Great Britain the number is considerably larger.

Experimental evidence that *Cooperia curticei* in very heavy infections (10,000 to 25,000 individuals) affects the economy of lamb production has been brought forward by some workers, notably Andrews (1).

There is a lack of experimental evidence of the effect of the parasites of the large intestine, but it is generally agreed that heavy infections with *Chabertia ovina* or *Oesophagostomum columbianum* are very harmful. Ross considers that more than 100 individuals of either of these parasites constitute a heavy infection.

For the purpose of comparisons for the preliminary work, the above findings and opinions were used for the adoption of tentative standards for conditions in Canada. Thus, infections which were considered harmful are:—

Haemonchus contortus	over 500 individuals
Ostertagia circumcincta	over 8,000 individuals
Trichostrongylus spp.	over 10,000 individuals
Cooperia curticei	over 10,000 individuals
Chabertia ovina	over 100 individuals
Oesophagostomum columbianum	over 100 individuals

In comparison with the above mentioned nematodes, all of which are commonly encountered in Canada, the other common parasites, *Moniezia expansa*, *Thysanosoma actinioides*, *Nematodirus* spp., *Strongyloides papillosus*, *Trichuris ovis* and *Muellerius capillaris*, were not considered as of primary importance but were always considered in subsequent observations. *Monodontus trigonocephalus* could not be placed in either of these groups owing to lack of data on its pathogenicity.

A large number of very poor lambs from the Eastern Provinces are placed on the market each year. From time to time viscera from such animals were obtained from abattoirs in order to collect specimens for identification, and in most cases a large number of nematodes or lesions due to the invasion of *Oesophagostomum columbianum* were present. It was thought that most of these animals were suffering from the effects of severe helminthiasis, although it was realized that observations made upon such animals would be of limited value without knowledge regarding other conditions, particularly those of nutrition, to which they had been subjected. However, in order to collect data on the limits of infection in these and in healthy stock from the same districts a number of observations were made. Lambs only were studied, and these were divided into "Cull lambs" which were thin and under 30 lb. dressed weight, and "Market lambs," which were healthy in appearance and over 30 lb. dressed weight. In the unhealthy lambs the dressed weight was, in most cases, somewhat less than 45% of the live weight, and in the healthy lambs it was usually slightly more than 50%.

The results of the examinations of these lambs are presented in Tables II, III, and IV. Each table represents animals killed during the same month, so that seasonal fluctuation of species need not be considered in the comparisons within each table.

It will be noted that the significant difference between the "Cull" and "Market" lambs in Table II (August) is in the number of *Haemonchus contortus* only. In Tables III and IV (September and October) this significant difference is not present, nor is there any other significant difference. This may be explained by the seasonal fluctuation of *H. contortus*, and would indicate that if any one species of parasite was responsible for the condition of the "Culls" it was *H. contortus*, the pathological effects having been produced earlier in the season.

TABLE II

LAMBS FROM LOTS SHIPPED FROM LUCEVILLE, BLACK LAKE, TROIS PISTOLES, NAPIERVILLE, STE. CHRISTINE, AND POINTE FORTUNE, QUE.
Killed in August, 1938

Lambs	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodules
"Cull"													
1	103	60	230	0	5	220	260	430	0	40	0	1	22
2	285	9,550	900	7	8	146	310	230	1,060	4	0	20	53
3	90	230	260	0	5	108	280	300	40	53	1	0	6
4	1,295	1,020	540	1	37	432	160	1,060	80	31	0	11	175
5	177	67	90	4	11	138	290	80	70	104	0	0	54
6	4,134	4,880	520	5	105	317	3,790	1,050	40	19	26	0	71
7	185	147	150	0	2	590	0	180	760	27	0	0	5
8	2,284	920	60	7	10	1,313	50	40	20	8	0	0	14
9	143	10,970	450	7	36	50	320	600	160	2	0	28	316
10	3,547	165	100	0	18	83	140	0	480	10	1	0	14
Totals	12,243	28,009	3,300	31	237	3,397	5,600	3,970	2,710	298	28	60	730
Means	1224.3	2800.9	330	3.1	23.7	339.7	560	397	271	29.8	2.8	6	73
"Market"													
1	19	940	360	0	73	22	90	50	140	89	0	7	138
2	3	780	540	3	16	15	320	120	60	9	0	7	184
3	241	330	80	8	12	26	0	2,770	0	2	5	0	117
4	30	303	80	0	4	258	30	280	40	2	6	0	8
5	188	621	0	0	47	36	0	80	30	0	0	0	1
6	21	334	80	0	3	66	40	30	20	22	0	0	6
7	57	1,172	630	14	23	324	10	260	70	27	7	0	13
8	23	60	160	0	6	116	50	250	400	2	1	0	11
9	20	566	0	0	7	59	40	140	0	7	2	0	0
10	651	1,470	460	90	4	1,210	270	900	0	17	11	1	32
11	280	410	100	16	21	349	0	250	50	16	1	0	6
12	3	10	10	0	0	0	0	0	0	1	0	1	40
13	21	30	10	2	2	19	0	0	0	5	1	0	23
14	494	2,020	0	0	0	1,280	300	320	0	4	0	0	0

TABLE II—*Concluded*

LAMBS FROM LOTS SHIPPED FROM LUCEVILLE, BLACK LAKE, TROIS PISTOLES, NAPIERVILLE, STE. CHRISTINE, AND POINTE FORTUNE, QUE. Killed in August, 1938—*Concluded*

Lambs	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodules
"*Market*" —con.													
15	462	500	30	0	0	60	20	50	0	6	0	0	7
16	469	230	90	0	0	30	0	100	0	12	1	1	10
17	269	261	160	2	2	23	10	30	20	9	0	1	34
18	157	237	0	0	0	1,070	0	0	0	39	0	0	2
19	10	123	120	0	0	8	0	0	0	9	0	2	49
20	138	3,010	4,360	16	0	30	120	2,180	0	5	0	8	147
21	76	34	0	3	0	15	0	40	30	2	0	0	37
22	980	340	30	0	0	32	20	10	20	2	0	0	13
23	45	780	350	0	13	32	30	90	250	8	0	0	14
Totals	4,657	14,561	7,650	154	233	5,080	1,350	7,950	1,130	295	35	28	892
Means	202.5	633.1	332.6	6.7	10.1	220.9	58.7	345.6	49.1	12.8	1.5	1.2	38.8

SAMPLE TESTS FOR SIGNIFICANT DIFFERENCES

Lambs	Parasite	Means	S.D.	Mean diff. ± S.E. diff.	P
"Cull"	*Haemonchus*	1224.3	1555.1	1020.9 ± 498.6	0.05
"Market"	*Haemonchus*	203.4	397.2		
"Cull"	*Ostertagia*	2800.9	4203.2	2167.5 ± 1331.0	0.1
"Market"	*Ostertagia*	633.4	354.6		

TABLE III

LAMBS FROM BEAUCE COUNTY AND MANIWAKI

Killed in September, 1938

Lambs	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodules
"Cull"													
1	188	1,100	0	0	63	34	20	50	450	6	25	1	4
2	21	2,200	460	12	11	320	160	4,950	0	51	14	0	61
3	428	100	60	5	35	20	200	120	20	1	16	6	116
4	2,014	940	290	18	134	42	1,150	3,180	450	3	3	32	34
5	1,276	1,350	940	35	52	24	90	1,590	80	5	5	6	454
Totals	3,927	5,690	1,750	70	295	439	1,620	9,890	1,000	66	63	45	669
Means	785.4	1,138	350	14	59	88	324	1,998	200	13.2	12.6	9	133.8
"Market"													
1	0	0	170	0	6	10	0	190	0	2	2	3	275
2	140	1,020	50	89	54	571	190	730	230	4	16	66	195
3	167	1,100	620	16	21	31	90	600	160	28	7	54	321
4	1,349	1,390	170	0	8	5	510	230	0	33	3	4	196
5	26	1,500	300	9	25	169	310	420	310	30	9	29	309
6	82	70	370	0	60	40	370	560	80	0	21	5	177
Totals	1,764	5,080	1,680	114	174	826	1,470	2,730	780	97	58	161	1,473
Means	294	846.6	280	19	29	137.7	245	455	130	16.1	9.7	26.8	245.5

TABLE IV

LAMBS FROM FRONTENAC COUNTY, QUE. (FOUR FLOCKS, CONTROLS FOR EXPERIMENTAL TREATMENTS)

Killed in October, 1938

Lambs	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodules
"Cull"													
1	1,414	2,650	4,190	7	53	75	10,950	4,060	280	10	5	25	1,613
2	130	1,500	2,630	12	32	60	2,720	3,790	220	1	0	2	1,299
3	138	610	1,820	5	99	430	4,330	810	230	1	1	3	641
4	240	890	1,440	10	8	30	3,600	2,380	60	11	0	2	1,561
5	226	1,920	3,190	10	43	60	3,310	720	200	11	0	24	1,630
6	130	950	3,990	6	28	210	1,780	350	80	9	0	5	748
7	186	660	1,280	5	57	30	1,310	530	220	1	0	5	726
Totals	2,464	9,180	18,540	55	320	895	28,000	12,640	1,290	44	6	66	8,218
Means	352	1311.3	2648.6	7.9	45.7	126.4	4,000	1805.7	184.3	6.3	0.9	9.4	1,174
"Market"													
1	140	730	2,600	6	16	20	1,030	2,290	60	4	0	4	1,431
2	109	270	1,340	12	33	310	3,340	1,160	240	13	0	6	1,040
3	152	560	1,870	12	32	10	1,840	1,250	110	9	1	2	1,379
4	792	1,370	4,380	9	175	0	850	1,720	440	11	2	19	930
5	105	510	3,340	0	33	38	900	2,710	50	33	0	5	1,098
6	68	270	5,970	19	17	30	2,140	1,760	680	12	1	2	1,003
7	40	80	4,190	0	5	0	210	1,440	0	11	1	1	169
8	110	250	3,180	3	25	20	80	440	0	13	1	0	50
9	727	260	430	10	5	0	50	560	70	44	0	0	154
Totals	2,243	4,300	27,300	71	341	428	10,440	13,330	1,650	150	6	39	7,254
Means	249.2	477.7	3033.3	8	38	47.6	1,160	1481.1	183.3	16.6	0.66	4.3	806

In order to support the above hypothesis, and for purposes of comparison with data obtained by Fallis (5) and other authors on seasonal fluctuation, further observations were made upon the incidence of the various species in a single flock. All the lambs from this flock were slaughtered as good market lambs, and the observations were made over a period of two seasons and thus on two crops of lambs from the same stock and on the same pasture. The data are presented graphically, each graph showing the observed fluctuations in the mean number of individuals of a species from July to March (Figs. 1–11). The number of animals observed was 275, being distributed as follows:—

July	27	October	54	January	14
August	33	November	24	February	15
September	93	December	8	March	7

Although the observations in some months were, of necessity, small, these graphs serve to illustrate the tendencies of fluctuations more accurately than data from mixed lots of lambs from widely separated districts. Such variations as the fall in the number of *Nematodirus* spp. in August, for example, cannot be accepted as true deviations from a curve, but the gross contours of the curves appear to illustrate the usual tendencies in eastern Ontario and Quebec, and agree closely with those compiled by Fallis in southern and western Ontario.

Notes on Parasitic Diseases of Lambs in Late Autumn and Winter in Eastern Canada

From the above data one would not expect to find verminous gastritis due to *H. contortus* or *O. circumcincta* in lambs in Eastern Canada during the late autumn or winter months. Nevertheless a large number of animals, from 7 to 11 months of age, are marketed each year during the cold months in extremely poor condition. Some are so emaciated that they weigh less than 20 lb. dressed, and are frequently held under observation by Federal Inspectors for mucoid degeneration. Table V shows the results of necropsies on sample specimens of these emaciated lambs, which were all less than 20 lb. dressed weight. These findings indicate two features of particular interest, the superinfection of *Trichostrongylus* spp. in three animals, the heavy infection of *Monodontus* in three, and the very large number of lesions due to *Oesophagostomum columbianum* in all the animals. These lesions, when present in large numbers, literally cover the intestines of small animals, frequently extending from the abomasum to the rectum. In very heavy infections there is little doubt regarding the pathogenic effect of the causative agent, particularly in those numerous cases in which partial or complete occlusion of the ileo-caecal valve has been produced. The number of lesions on the small intestines is approximately the same as on the large intestines, but the region around the ileo-caecal valve appears to be constantly filled with nodules.

Owing to the fact that the effects of infection by *Oesophagostomum columbianum* in lambs in Eastern Canada appear to differ in several important details from those in other parts of the world, this disease has been the subject

TABLE V

EMACIATED LAMBS FROM EASTERN CANADA SLAUGHTERED IN DECEMBER AND FEBRUARY

Month of slaughter	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodular lesions
December	0	0	3,310	42	49	0	490	12,140	0	10	27	17	1,008
	60	190	60	4	214	0	150	740	0	7	0	20	905
	0	10	1,910	0	324	70	9,140	10,150	0	776	36	4	541
	0	60	2,340	10	23	200	1,130	3,530	50	29	6	3	704
	0	50	2,450	1	27	0	1,820	8,830	?	9	12	13	931
	10	210	1,020	2	28	0	1,820	4,030	150	4	16	7	1,119
February	0	40	4,000	1	197	210	2,550	2,010	?	0	3	104	306
	19	760	2,800	0	.22	0	0	5,700	?	4	0	468	1,682

of special studies, the results of which will be reported in a later paper. As the position of the lesions on the intestinal tract appears to exert a greater influence on the health of the host than mere numbers, a tentative line between tolerated and harmful infections is difficult to set. It is apparent, however, that less than 300 lesions do not affect the normal development of the young animal, and that under certain conditions a well grown lamb can tolerate a much larger number.

The Parasites of Adult Stock in Eastern Canada

Because of the methods of marketing sheep in Canada, whereby the young stock is sold as "lamb" from July until the following spring at from 4 to 11 months of age, it is difficult to obtain viscera from relatively young breeding stock for parasitological studies. These marketing practices are due to economic necessity, because the cost and labour of overwintering any sheep other than those to be used for breeding could not be recovered from future sales. Thus a suitable market for "mutton" has not been developed, and adult sheep are usually marketed only when they have become unprofitable to their owners and must be sacrificed at a low price. Sheep which appear on the markets in the spring, early summer and in the winter are frequently in poor condition, and the majority are more than three years old.

Adult sheep in Eastern Canada are almost invariably infected with lesions due to *O. columbianum*; during the course of these studies only two lots of sheep from the Province of Quebec that included individuals free from this infection have been seen. These lots came from Lac St. Jean and Amqui, two more northerly points of agricultural Quebec; all other lots have shown numerous lesions in all individuals.

During the course of field investigations, small numbers of ewes have been killed and examined on farms in attempts to determine the cause of emaciation and inability to nourish their lambs. Severe infection with lesions of oesophagostomiasis have been the only constant pathological process noted. Samples of emaciated ewes placed on the market in Montreal during the spring and early summer have been examined. The number is too small for any definite decision regarding the cause of the emaciation, but, in the absence of any obvious pulmonary infection, the findings in regard to helminthic infections are of some interest. The results of necropsies on eight ewes over four years of age are shown in Table VI. These findings appear to indicate the probable effect of the numerous lesions of oesophagostomiasis in the presence of only light infections of other helminth parasites in the intestinal tracts.

The Parasites of Sheep and Lambs from Western Canada

Large numbers of lambs from the ranges in southern Saskatchewan and Alberta are slaughtered in Eastern Canadian abattoirs each year. These animals are usually excellent fat lambs of the Rambouillet breed. The most striking feature noted during the abattoir operations is that these animals are entirely free from lesions of oesophagostomiasis. There is, however, a

TABLE VI

EMACIATED ADULT EWES FROM EASTERN CANADA

—	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*	*Oesophagostomum*	Nodular lesions
1	0	4	97	0	13	0	12	8	?	0	0	3	1,180
2	0	16	50	0	8	10	40	110	?	0	0	10	1,088
3	2	10	70	0	8	0	0	20	?	0	0	0	520
4	0	10	310	0	5	10	0	30	?	0	0	0	1,685
5	0	7,900	4,720	0	45	50	0	210	?	0	1	6	887
6	0	1,750	2,850	0	52	150	70	300	?	0	69	48	760
7	194	460	950	0	12	0	170	10	?	0	0	17	543
8	10	150	0	0	23	0	340	0	40	0	0	0	1,099

fairly high incidence of infection in the intestine and bile ducts with the Fringed Tapeworm, *Thysanosoma actinioides*. A sample of 18 animals from a "feed-lot" in western Ontario, which had been received previously from southern Saskatchewan, was examined in 1934. All these animals were infected with *T. actinioides*; although tapeworms were present only in the bile ducts of five livers, the majority were in the small intestines. Very light infections of both *H. contortus* and *O. circumcincta* were present in all the abomasa, but the greatest number of these stomach worms in a single lamb was 30. Fourteen of the small intestines contained small numbers of *Nematodirus filicollis*, 12 contained small numbers of *Trichostrongylus colubriformis*, and six contained up to ten *Monodontus trigonocephalus*. All the large intestines contained small numbers of *Trichuris ovis* and two contained up to five *Chabertia ovina*. In addition to the above, one specimen of *Moniezia expansa* and two of *Capillaria longipes* were recovered from the lot.

The only infection of interest in these fat lambs was that of *T. actinioides*; 100 of these parasites were present in one small intestine and two were in the liver of the same animal.

During these studies the abattoir operations on several hundreds of fat western lambs have also been observed and a high incidence of *T. actinioides* in the bile ducts has been noted. These observations have led the writer to agree with Christiansen (3) that this parasite is not of major importance.

Viscera from western sheep and lambs that were reputed to be suffering from parasitic disease have been examined from time to time. The results of these examinations are shown in Table VII, the numbers of parasites in all but the last four animals being approximate.

The animals from Saskatchewan and Alberta were examined for the purpose of routine diagnosis and the numbers of individual parasites were not accurately recorded. However, in no case was an infection of pathological significance noted. The cases from British Columbia are very interesting. The animal from Agassiz was pastured on land which is subject to a very high summer rainfall and relatively high summer temperatures, and was undoubtedly suffering from haemonchiasis. In addition, the number of hookworms present was of probable clinical significance.

The three lambs from Vavenby had been subjected to entirely different conditions, and had ranged over land in the drier interior of the province. They were samples of a number of animals that had suffered from an enteritis characterized by scouring, with dark fetid faeces. The diagnosis in these three cases was severe chabertiasis, extensive damage having been produced in the crown of the small colon. These cases are of particular interest because they constitute definite evidence of the occurrence of a parasitic disease in a part of the Dominion that is of major importance in sheep husbandry. The number of individuals of *Chabertia ovina* present in each animal appears to be greater than any cases recorded from other countries.

TABLE VII

PARASITES IN THE INTESTINAL TRACTS OF UNHEALTHY SHEEP AND LAMBS IN WESTERN CANADA

Animal	Origin	*Haemonchus*	*Ostertagia*	*T. axei*	*Moniezia*	*Thysanosoma*	*Monodontus*	*Nematodirus* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*S. papillosus*	*T. ovis*	*Chabertia*
Lamb	Edmonton, Alta.	0	50	10	0	2	0	1,539	40	0	0	3	0
Lamb	Edmonton, Alta.	0	410	0	0	0	0	1,149	30	150	0	0	0
Lamb	Tilly, Alta.	50	400	?	1	0	0	Few	Few	Few	?	5	0
Lamb	Tilly, Alta.	200	2,000	?	Few	0	0	700	12	?	?	12	7
Lamb	Cypress Hills	150	75	?	0	0	0	30	?	?	?	0	0
Lamb	Hatton, Sask.	0	Few	?	3	0	0	130	?	Few	?	0	0
Lamb	Piapot, Sask.	10	Few	?	10	0	0	40	?	?	?	1	0
Ewe	Maple Creek, Sask.	40	14	?	1	0	0	40	?	?	?	0	0
Ewe	Agassiz, B.C.	1,487	4,880	520	5	0	105	317	3,790	1,050	40	19	26
Lamb	Vavenby, B.C.	0	1,750	830	0	0	0	?	?	?	?	30	610
Lamb	Vavenby, B.C.	0	7,950	2,390	4	0	0	2,080	800	600	?	7	682
Lamb	Vavenby, B.C.	0	3,420	3,510	3	6	0	2,500	3,010	3,190	?	75	658

Discussion

In these preliminary studies an attempt has been made to show which species of parasitic worms are commonly present in sheep and which are of major importance. Data have also been presented in order to explain causes of unthriftiness in groups of animals, chiefly from Eastern Canada. From these data it is apparent that verminous gastritis, due to infection with *Haemonchus contortus*, is an important disease of lambs during the grazing season in the Province of Quebec and probably in all parts of Eastern Canada.

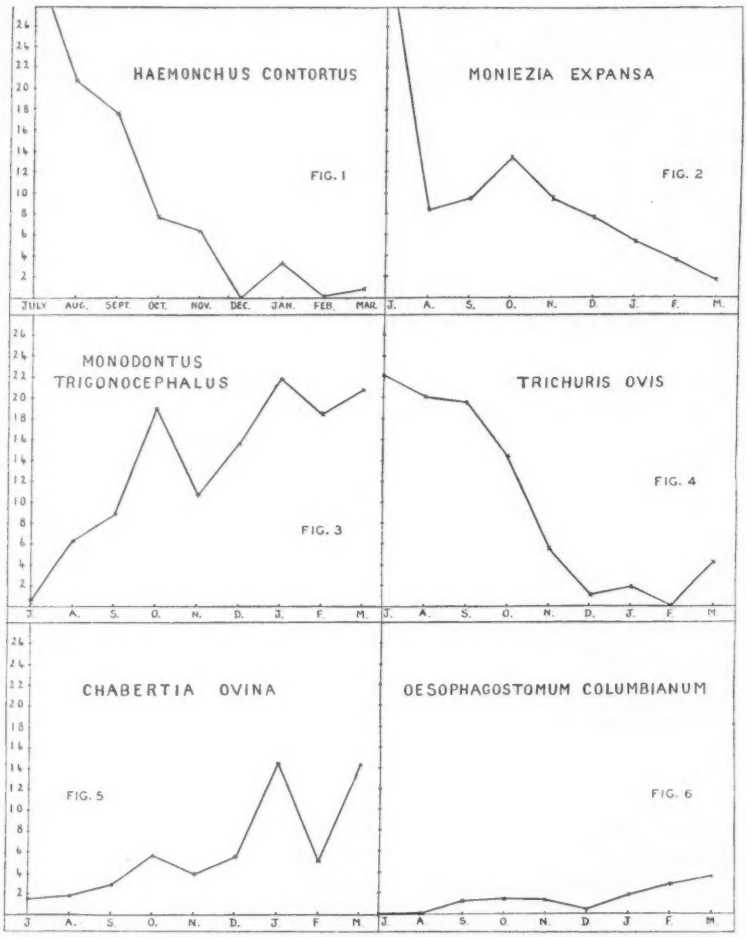
Infection with *Ostertagia circumcincta* has not been found of major importance, but, having reference to findings in Great Britain and elsewhere, this paper has not shown that this parasite cannot become of importance under certain conditions during the summer.

In the autumn and winter months in Eastern Canada verminous gastritis becomes of lesser importance, but heavy infections of *Trichostrongylus* spp. and the lesions due to invasion of the intestinal walls by larvae of *Oesophagostomum columbianum* become the apparent causes of unthriftiness.

In adult sheep in Eastern Canada the lesions of oesophagostomiasis appear to be a cause of marked unthriftiness during the breeding season.

The nature of parasitic disease in sheep in the Prairie Provinces has not been made apparent. In British Columbia records of importance have been made; in one region which is subject to a high summer rainfall, haemonchiasis and possible hookworm disease were recorded, and in a drier region in the interior of the province diagnoses of severe chabertiasis were made.

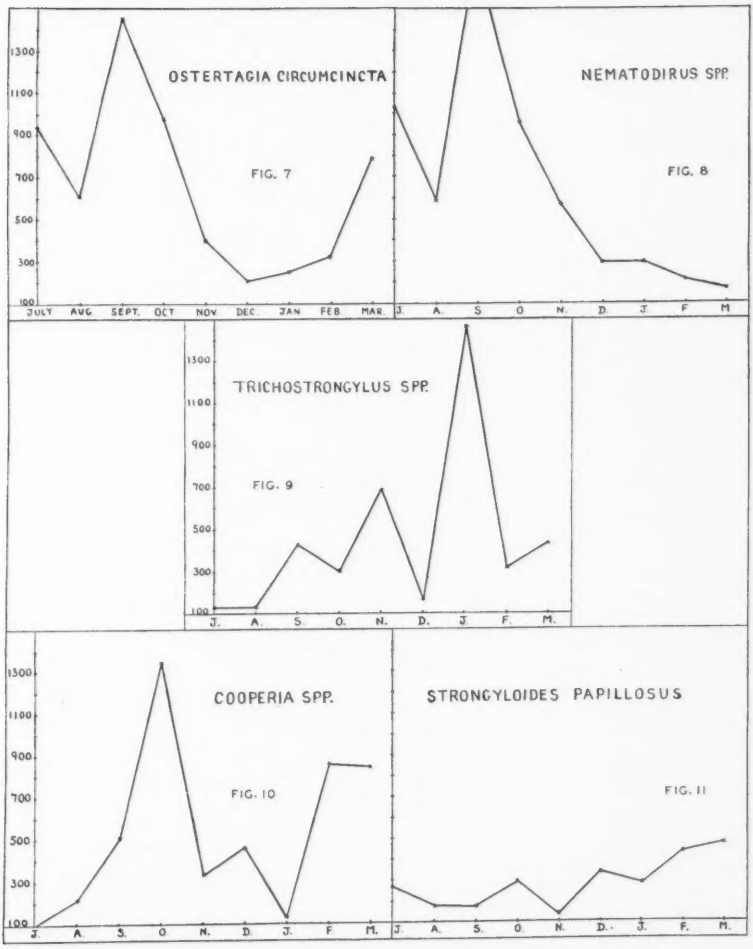
These preliminary surveys were made necessary by the fact that certain species of parasites that are of known pathogenic importance in one region may be present but of little or no importance in another region which is subjected to a different climate and different methods of husbandry. The pastures in Eastern Canada are not used during the winter months, when they remain under snow for a long period. Griffiths (6) has shown that *Moniezia expansa*, *Ostertagia circumcincta*, *Nematodirus filicollis* and *Trichostrongylus colubriformis* are able to survive a normal winter on a pasture in the Province of Quebec. If the free-living stages of a certain species do survive and are present on the pastures in the spring, then one might expect this species to be present in reasonable numbers in lambs after they have grazed on the pasture for from six to eight weeks. Figs. 1 to 11 show the relative incidence of the common species in July, and would indicate, according to the above hypothesis, that *M. expansa*, *T. ovis*, *O. circumcincta*, *Nematodirus* spp. and *H. contortus* survived the winter. Accordingly, then, it is also apparent that winter survival in the case of *M. trigonocephalus*, *C. ovina*, *O. columbianum*, *Trichostrongylus* spp. and *Cooperia* spp. is low or non-existent. It will be noted, however, that *H. contortus* was present only in abnormally small numbers in these animals, and is numerically comparable to five other species that are usually present in small numbers (compare with data obtained from



FIGS. 1–6. *The seasonal fluctuation in numbers of the species of helminth parasites in the alimentary canal of lambs in Eastern Canada.*

market animals). Owing to this abnormality, which is probably due to the fact that the flock was treated with anthelmintics known to have a positive action upon *H. contortus*, this species cannot be considered in the hypothesis regarding winter survival.

The factor of non-survival on the pastures, combined with factors of summer rainfall and temperature, would have a profound effect upon the ability of certain species to be of pathogenic importance. These preliminary studies have, then, demonstrated the importance of knowledge regarding the biology



FIGS. 7–11. *The seasonal fluctuation in numbers of the species of helminth parasites in the alimentary canal of lambs in Eastern Canada.*

of the free-living stages of each species of helminth parasite present in sheep in Canada.

It is already apparent that owners of sheep in Eastern Canada must adopt efficient means of combating haemonchiasis in their animals during the summer months, and that they have urgent need of some measures of control of oesophagostomiasis in order to protect the health of breeding stock. The possible occurrence of disease due to heavy infections of *Trichostrongylus* spp., *M. trigonocephalus*, and *C. ovina* must always be considered.

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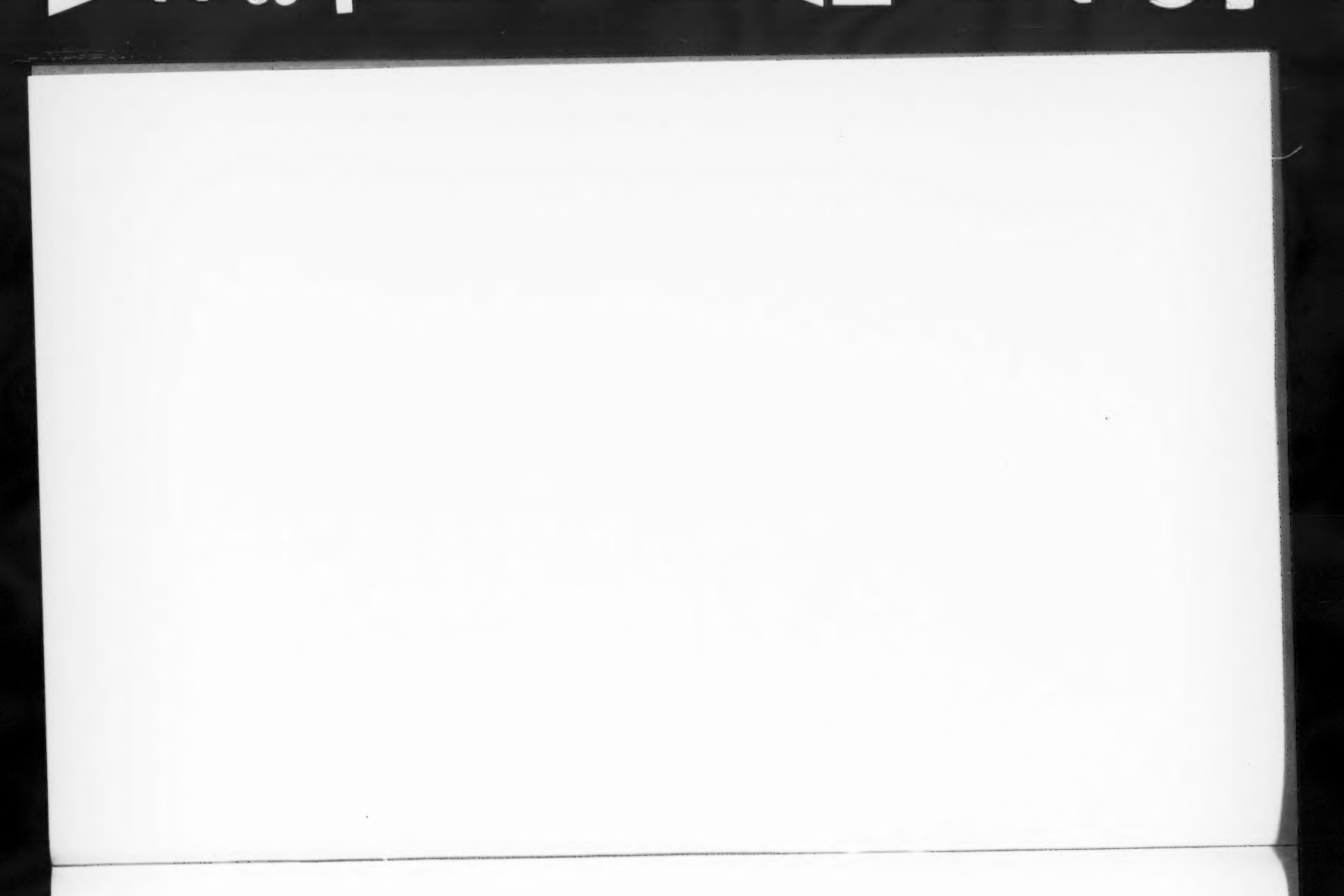

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